

What to pay for nuclear power?

A House of Commons committee has thrown new light on the ways in which British governments too often make bad decisions, especially where technical matters are concerned.

POOR (in both senses) Britain is paying a heavy price for the incompetence of its public administration, especially of technical matters. That is the chief message of the splendid report from the House of Commons Select Committee on Energy published last week (see also page 7). Ostensibly, the committee set out to tell why the nationalized electricity utility in England and Wales called the Central Electricity Generating Board (CEGB) should have told a public inquiry in 1987 that the price of electricity from a newly constructed pressurized water reactor (PWR) would be 3.09 pence per kilowatt-hour, and should have been quoting prices more than twice as much when negotiating with the government the terms on which it should be privatized. Readers of the report will not be better able to name a figure for the cost of nuclear electricity, but they will have a better understanding of the vacillations of government policy on nuclear energy.

It is a bizarre tale. Since 1972, the CEGB has been seeking to build PWRs, on the grounds that they could be built more quickly and would be intrinsically more economical than the gas-cooled reactors it had inherited from Britain's earlier flirtation with nuclear power. In 1979, the incoming Conservative government said it would sanction a programme to build ten PWRs over some forthcoming decade, but was then constrained by its predecessor's decision that none would be built until there had been a public inquiry. By the time the construction of the first reactor had begun in 1987, the government had also decided that the electricity industry should be "privatized". But it failed to appreciate that its nuclear policy and privatization policy might be in conflict. Now there is a gigantic muddle.

What the energy committee has most usefully done is to throw light on the casual ways in which these policies were made. The plans to privatize the electricity industry were made public in February 1988 by the then Secretary of State for Energy, Mr Cecil Parkinson, without a serious attempt to tell whether they were feasible. On the generating side, CEGB was to be split into two parts, 'Big G' and 'Little G' (now National Power and PowerGen respectively), but in the face of the CEGB's protests that nuclear power would be a problem.

So it has proved to be. First, in July last year, the government was forced to concede that the older gas-cooled reactors would have to remain in public ownership: reactors nearing the ends of their lives, but saddled

with huge liabilities for decommissioning, would hardly have been an attractive investment. But then, last November, it had to go the whole hog, and lump all nuclear generating capacity into a third company, called Nuclear Electric, that will remain in public ownership.

The fascinating argument between the CEGB and the government, now revealed, about the price of nuclear electricity has been a proxy for the Department of Energy's failure to understand what privatization would entail. Should the discount rate (the notional cost of money) be 8 or 10 per cent? Is it surprising that CEGB, when told to ask British Nuclear Fuels (ironically, publicly owned) for a fixed price for reprocessing fuel, should be quoted a price 30 per cent greater than expected? Or that the CEGB should want to amortize new reactors over 20 years (rather than the full 40 years of life expected) to allow, in part, for the political risks ahead? In the end, the government took the view that the CEGB was asking for too much, and said no.

This sorry sequence of events may have permanently queered the pitch for nuclear power in Britain, and will also have played havoc with the public accounts. Nuclear Electric will operate existing reactors with the help of a subsidy collected from electricity consumers and called a Fossil Fuel Levy. (Britain will thus be the first industrialized country to have a carbon tax.) The Energy Committee is right to demand that the fixing of this charge should be transparent. Further nuclear construction has been postponed until a review in 1994. The Energy Committee is right to insist that the outcome of that review should be publicly debated before future policy is made. Meanwhile, there is a PWR half-built in Suffolk whose future is uncertain; why not finish the plant but not load it with fuel? That would avoid decommissioning costs and provide a lasting monument to four decades of mismanagement. □

Hubble trouble

The defects that have come to light in the Hubble Telescope are a serious setback for research.

THERE was always a risk that the Hubble Telescope, already more than three years late, would have been lost during the tricky process of putting it into orbit. That it should turn out to be incapable of being focused



accurately is not much better (see page 3). It hardly matters that there will now be a grand inquisition to tell who is to blame, or that the US National Aeronautics and Space Administration (NASA) believes that an astronaut may be able to fix the trouble in 1993. The plain fact is that a decade's work by hundreds of skilled researchers has been undercut by an elementary error. Some hope that useful science can be salvaged, but three years out of an expected lifetime of fifteen are one loss, the inevitable degradation of many Hubble instruments another.

What should happen now is what matters most. Even if there were funds in the space science budgets of NASA and the European Space Agency (a 15 per cent partner in the venture), building another Hubble and relaunching it would not serve a useful purpose. The design of the instrument has its origins more than a decade back, while something else might in any case go wrong next time. Beginning now on the design of an even better instrument might mean that there was something to show for the effort early next century, but that is a long time to wait. By then, in any case, the design and construction of terrestrial telescopes will have reached the point at which only a radically better successor to Hubble would be worthwhile.

Last week's disappointment points sadly in only one direction — that Hubble loaded too many of the research community's eggs in the same basket, and a frail basket at that. Even if Hubble had been launched successfully, the intermittency of seeing caused by the Earth's shadow and the difficulties of planning the ponderous reorientation manoeuvres of the telescope to make the best use of its expected life, however splendidly accommodated by the software engineers, are a proof that an instrument as sophisticated as this deserves a much higher orbit. But that would mean extra power for data transmission, and an even more elaborate package . . .

It will nevertheless be disastrous if this setback means that high-resolution astronomy from orbit is now abandoned, or postponed until after 1993. Low-resolution satellite astronomy in other than the visible regions of the spectrum has been a great success. The forthcoming ROSAT X-ray telescope will have greater resolution than its predecessors, though it has been much delayed. But there is an urgent need for complementing ground-based observations in the visible region with greater resolution from beyond the atmosphere. The need is for much simpler instruments in which versatility and optical speed are sacrificed for resolution and which are, perhaps, designed to answer predetermined questions. One virtue of instruments in orbit is that their users are not forced to hurry. They can sit there for days on end, collecting light from single objects. It would not be out of order that some imaginative agency should seek to lift the present gloom by running a competition for sufficiently robust designs. After recent experience (not only of Hubble) they should be capable of being launched by old-fashioned rockets, not through the bottleneck of the shuttle, which has now been thoroughly discredited. □

Himalayan clouds

The expedition to test Professor V. J. Gupta's claims is in doubt; Chandigarh should take other steps.

THE Panjab University of Chandigarh should not be too disconcerted that Professor V. J. Gupta has been taken ill and that the expedition he was to lead to some of the disputed fossil-sites in the Himalayas will probably not now set off in the next few weeks. Gupta's inability to travel has understandably prompted other members of the proposed expedition to say they will not go either. But the notion of testing the veracity of a whole corpus of Himalayan palaeontology by collecting fresh material from places and strata nominated by Gupta was never a particularly good one, anyway, at least without much more careful preparation than the proposed expedition had been given.

The essence of the doubt cast on much (not all) of Gupta's work is that some of the fossils he has described are unlikely to have come from the places in which he said he found them. Verification is complicated by the vagueness of the descriptions of locations in the published reports. (Sadly, Gupta is not the only palaeontologist to have erred in that respect.) A well-planned expedition would have begun with an attempt, independently of Gupta, to identify a few among the disputed fossils for which verification could be expected to be at once feasible, straightforward and in some sense clinching. Then the expedition would have known what it was looking for and, broadly speaking, where. It would not have been a particularly comradely expedition, of course; Gupta, ostensibly the leader, would have felt an uncomfortable pressure to deliver the goods under the eyes of watchful, even sceptical companions. But in the event, very little thought seems to have been given to the planning of the expedition except that it should set off some time this northern summer.

Dr R. P. Bambah, the vice-chancellor at Chandigarh, has rightly said that he wants neither a "witch-hunt" nor a "whitewash". No scientist whose work has been as seriously questioned as has been Gupta's deserves less of his university. But there are simpler procedures on which the university could and should fall back. By now, there have been such specific complaints against some of Gupta's conclusions that an expedition of verification need travel no further than his laboratory. Some of the disputes that have arisen could probably be settled if Gupta were asked to furnish a group of independent palaeontologists with original specimens (or to explain why they do not exist). The question is not whether there are, for example, conodonts in some Himalayan formations, but whether Gupta's past published reports of them are sustained by the evidence. Going off to the mountains is neither a necessary nor a sufficient test of that. What Chandigarh must recognize is that its own reputation will suffer from further delay. □

Hubble Telescope has misshapen mirror

■Non-imaging work to be emphasized

■Corrective lens by 1993?

Washington

THE Hubble Space Telescope (HST), its builders have proudly claimed, contains the most precise optical system ever made, with reflecting surfaces polished to an accuracy of less than one-fiftieth the wavelength of light. That claim is still true.

But unfortunately, as officials of the National Aeronautics and Space Administration (NASA) admitted last week, the optical system was precisely made to the wrong shape, making HST's resolving power the same as that of the best ground-based telescopes, not ten times better.

But HST astronomers are confident that much of the science programme can be salvaged, and the defect is simple enough that replacement cameras equipped with corrective lenses, which could be installed by a space shuttle crew three years from now, will restore HST's full acuity.

The flaw was discovered as NASA engineers began to adjust the position of HST's secondary mirror, a small convex mirror that reflects light from the 2.4-m concave primary into the scientific instrument package. By shifting the secondary up and down the axis, astronomers expected a stellar image to come into perfect focus.

But the image could not be focused, and analysis of the way it changed as the secondary was moved indicated that HST suffers from spherical aberration caused by a departure of the radial profile of one of the mirrors from the required shape. The defect was such a "textbook example", according to deputy project manager Jean Oliver, that it must have been ground into the mirror during manufacture.

The departure of the optical surfaces from the required shape is small — about half a wavelength of light — but enough substantially to reduce the image quality (see below).

Most planned HST observations requiring high-resolution imaging will be put aside, but NASA officials were anxious, when announcing their predicament last week, to emphasize the "unique and important" science that HST can still do. Project scientist Ed Weiler pointed out that three of the HST instruments, the high-speed photometer, the high-resolution spectrograph and the faint object spectrograph, are non-imaging devices and had been expected to do much of their most significant work in ultraviolet regions of the spectrum, which are inaccessible from the ground.

The loss of resolution means that observations of faint, finely detailed objects

will generally not be feasible, and HST managers will replace such observations with proposals that had been turned down for lack of available time.

Weiler, along with deputy project manager Jean Oliver, was hopeful that HST's spherical aberration can be so exactly diagnosed that it will be possible to design a simple corrective lens to restore HST's full ability. An improved Wide Field/Planetary Camera is in fact already partly built, and with corrective lens added could be installed during a scheduled shuttle flight as early as June 1993.

The same can in principle be done for the Faint Object Camera, but Peter Jakobsen, HST project scientist for the European Space Agency (ESA), which provided the camera, said that no specific plans could yet be made.

While astronomers were mostly interested in finding remedies, NASA is setting up a review panel chaired by Lew Allen, director of the Jet Propulsion Laboratory, to determine the cause of the defect. Oliver was adamant that HST's optical design was correct, and Bob O'Dell of Rice University, who chaired a space telescope planning group in the 1970s, agreed that the design had been checked by so many people that any possibility of a flaw had been eliminated. The error, Oliver concluded, must therefore have been in the mirror testing procedure.

The primary and secondary mirrors were never tested in combination. According to Oliver, a test of the optical system of HST would have required the construction of an optically flat surface as big as the primary; the cost would have run into hundreds of millions of dollars. Instead, the two mirrors were separately tested for conformity to the desired profile. Somehow, during the manufacture and testing, one of the mirrors was made to the wrong shape, and it will be the job of the review panel to discover how an error that should easily have been caught remained hidden until three weeks ago.

That job may not be easy. HST's mirrors were completed in 1981, and few of the people now involved in the project have any connection with those early days.

When NASA put construction of the HST optical system out for bids, the Perkin-Elmer company, an experienced builder of high quality optics, sent in a proposal that included a detailed account of how the optical surfaces would be separately finished to NASA's specifications. Although, as O'Dell recalls, some astro-

...and the good news

THE optical system of the Hubble Space Telescope was designed to put about 70 per cent of the light from a point source into an image spot 0.1 of an arc second in diameter. Atmospheric turbulence prevents the best ground-based telescopes from producing images smaller than about one arc second.

A photograph made on 20 May by HST's Faint Object Camera (FOC) resolved stars into a central bright spot less than 0.1 arc second across and containing about 20 per cent of the light, the remainder being spread across a one arc second disc. It now turns out that FOC's first shot is as good as it will get.

At first, NASA officials and astronomers emphasised HST's capacity to do non-imaging photometry and spectroscopy, especially in the ultraviolet, where HST's chief advantage is simply to be above the Earth's atmosphere. But in the days following the announcement of the flaw, some have become more optimistic.

Peter Jakobsen of the European Space Agency, which built the FOC, says that because HST's images have a prominent core surrounded by a fainter halo, images of high-contrast objects, such as densely packed stars at the centre of globular cluster, will still be informative. And the fact that HST's distortion is of a constant, wavelength-independent form means some computer image-reconstruction techniques may be useful.

On the other hand, HST will not be able to see extended objects of low surface brightness any better than ground-based telescopes. It had been hoped, for example, that the FOC would be able to photograph the faint galaxies in which distant quasars are thought to reside, but Jakobsen now thinks the quasar light that spills into the one-arc-second halo will overwhelm any galactic light that might be there.

Nevertheless, Jakobsen thinks NASA was too "downbeat" in saying that virtually all the imaging work of HST will be useless, and emphasized that it is "early days" for astronomers thinking of ways to compensate for HST's deficiency. **D.L.**

nomers at the time would have been happier with an integrated test of the optics, Perkin-Elmer's proposal was judged adequate to the task.

Perkin-Elmer (now Hughes-Danbury after a take-over) won the contract and built the mirrors, but project managers at NASA's Marshall Space Flight Center (MSFC) in Huntsville, Alabama, had responsibility for ensuring that the terms of the contract were fully carried out. The review panel will reconstruct what happened both at MSFC and at Perkin-Elmer to find out how the faulty mirrors passed muster at both places. **David Lindley**

Giotto steals a ride

Munich

THE Earth lost a minute fraction of its orbital velocity earlier this week as the European Space Agency (ESA) Giotto space probe stole a little of the Earth's energy to help it on its way to a rendezvous with the comet Grigg Skjellerup. It was the first time the manoeuvre, the same "gravity assist" that helped the NASA Voyager probe on its journey from Jupiter out to Saturn and Neptune, had been executed using the gravitation field of Earth. According to ESA researcher Trevor Morley, Giotto will gain 3.1 km/sec in velocity, meaning that the Earth will slow in its orbit by one millimetre in every million centuries.

A free ride on the Earth's gravity was a welcome break for a project that is beset by funding uncertainties. Despite the tremendous success of the original Giotto mission to Comet Halley in 1986 (see *Nature* 320, 202 & 321, 259; 1986) and the potential for further cometary exploration, ESA voted last month to make the Giotto Extended Mission (GEM) an optional, not mandatory, project within its science programme. Member states will meet in September to discuss whether they are prepared to pay for Giotto.

Project manager Manfred Grensemann at the ESA ground station in Darmstadt is "optimistic" that enough support can be found. He estimates that the next phase of the mission would cost 10 million ECU (about \$8 million), mostly for the communications needed to keep in touch with the probe as it moves farther away.

"Compared to starting a new mission,

this is a bargain", says GEM project scientist Gerhard Schwehm. Schwehm said that the second flyby would be a unique opportunity to expand knowledge of comets. It will be especially interesting, he said, to study the interaction of the coma of the comet and the solar wind, as well as to compare the distribution and optical properties of dust around Comet Grigg Skjellerup with that around Comet Halley, which had at least 100 times more of it.

Giotto was one of five spacecraft to approach Comet Halley in 1986, but it moved far closer than the others and came away with dazzling photographs of the nucleus. After the Halley encounter, Giotto was put into 'hibernation' to conserve power as researchers sought a suitable second comet to study with the craft. The probe will now be put into hibernation again until May 1992, when it will 'awaken' and prepare for the next encounter.

Unfortunately, said Grensemann, the camera that took the dramatic shots of the nucleus of Comet Halley is no longer operating — the sensors still function but no light reaches the aperture. But seven other instruments on board are at least partly operational and Grensemann expects them to deliver good data.

The researchers chose Comet Grigg Skjellerup as Giotto's target as much for practical as for scientific reasons. The comet was in a position in its orbit around the Sun that meant only minor adjustments in the trajectory of Giotto were needed for them to meet.

Steven Dickman

RHEUMATOID ARTHRITIS

New institute for Berlin

Munich

A RESTORED villa on the Kleiner Wannsee, a lake within West Berlin city limits, will become home for a new institute for research into the causes of rheumatoid arthritis. Immunologist Avron Mitchison of University College, London, has been named as scientific director and will work in Berlin half-time.

As a result of a political squabble over the institute between the left-wing coalition of Social Democrats and Greens which took office last year in West Berlin and the Conservative Christian Democrats who begin the project, the institute will study not only the molecular biology of the disease but will also look at "psychosocial factors" that contribute to its development. Mitchison says that this area has been neglected in rheumatoid arthritis, especially in comparison to the attention it has received in cancer.

Steven Dickman

CONFERENCES

Changes at Dahlem

Munich

SILKE Bernhard, scientific director of the Dahlem Conferences, is to step down because of ill health. A physician by training, Bernhard had been credited by participants with building the high international reputation of the conferences.

Last year, Bernhard struggled to maintain the conferences when their original backer, the 'Donors Association for Promoting Arts and Sciences in West Germany' (*Stifterverband für die Deutsche Wissenschaft*) withdrew its support. The Free University of Berlin took over sponsorship in November (see *Nature* 340, 86 & 342, 466; 1989).

Jennifer Altman, a neuroscientist and formerly on the staff of *Nature*, will act as interim scientific director until an international search can be arranged for a permanent director.

Steven Dickman

Murmurs of complaint

Washington

JAPAN'S national university professors are all employees of the government, which puts them in an odd position when they want to protest to the government about university conditions. But last month, the Association of National Universities finally succeeded, after years of trying, in winning funds from the Ministry of Education, Culture and Science to set up a committee to study their own financial difficulties.

The association represents all the 93 national universities directly supported by the government and has responsibility for setting up the general entrance examination.

Akito Arima, president of the association and of the University of Tokyo, recently described the universities' difficulties in a magazine of the Ministry of Finance. "In Japan about 1 per cent of GNP is allocated to college education and research, but in the United States it is 1.5 per cent. Within the limited budget, priority is placed much more on building new laboratories, moving universities, and big science, and less on the reconstruction or the maintenance of existing laboratories.

"As a result, buildings and equipment are becoming old and dilapidated. Good research cannot be produced by laboratories that are too small and lack both good equipment and money. We must find other financial sources." The new committee has not decided its plan of action and will first gather basic data.

Shigeko Segawa

ANTARCTICA

Airstrip plans on ice

Sydney

AUSTRALIA has been forced to abandon plans to build an 'environmentally friendly' A\$1 million airstrip close to its Casey Base in Antarctica. The experimental runway was built on ice to minimize the effect on wildlife, most of which lives on the very small part of the Antarctic that is rock. Construction of a rock runway at the French Antarctic base led to an international outcry as Greenpeace claimed that an important breeding ground for Adélie penguins had been destroyed.

But ice has not proved to be safe after all, according to a report by the Royal Australian Air Force. Last summer, the report says, a vehicle broke through the ice on the runway and sank into the water beneath. Because of the risk of the runway melting and breaking up, the air force argues that a gravel airstrip should be built.

The Australian Department of the Environment's Antarctic Division has proposed an alternative site for an ice and snow runway 30 km away from Casey where the climate is harsher. The United States has a successful ice strip at its McMurdo base south of Casey.

Tania Ewing

First test of incineration

Boston

If all goes as planned this week, the US Army will destroy by incineration a first batch of chemical weapons at its new facility on remote Johnston Island in the Pacific Ocean. The trial incineration, which the Army calls "operational verification testing", is the first step in fulfilling the agreement between US President George Bush and Soviet President Mikhail Gorbachev earlier this year to destroy the bulk of the superpowers' vast chemical arsenals (see *Nature* 345, 192; 1990).

Although the chemical weapons agreement was widely welcomed, both sides acknowledge that destroying the weapons presents environmental dangers that could engender considerable political opposition. On the US side, the Johnston Island facility represents the US Army's attempt to show that the ageing chemical weapons can be disposed of safely. The incinerator was completed last year, before the recent agreements, to destroy a large stockpile of chemical weapons that had been stationed in the Pacific during the Second World War. These weapons, amounting to several thousand tons, have been stored since 1971 on Johnston Island, which is some 700 miles from its nearest Hawaiian neighbour.

Army chemists say that because the chemical components of the nerve, mustard and blister agents to be burned are known so precisely, and because of the facility's state-of-the-art design, it should generate fewer toxic emissions than conventional trash incinerators. But this optimistic view is not universal. Dozens of environmental groups have objected to the facility during the lengthy political process required for it to open, decrying the potential air pollution and toxic solid wastes generated by incineration and favouring alternative disposal methods.

Even as testing begins, Sebia Hawkins of Greenpeace says that there is "still hope that the facility can be stopped". But environmentalists concede that the Army has largely sidestepped opposition because the island is so remote and lacks a native population. Future incinerators to handle the vast bulk of the nation's stockpiles remain to be built, and are likely to generate far more political opposition.

Almost everyone involved in the project admits that a deadly accident could occur. As carefully designed as the handling procedures are, the sheer scale of the operation is worrying.

The Johnston Island facility will handle thousands of tons of lethal chemicals — some of which can kill in microgram quantities — and burning all the chemical agents now stored on the island is expected to keep the incinerator running steadily for over a year.

Adding to the controversy is the fact that later this year another 430 tons of chemical weapons are scheduled to arrive at the island. The new intake comes from West Germany in the wake of an agreement in 1987 between then US President Ronald Reagan and German Chancellor Helmut Kohl that the United States would remove its chemical stockpile from German soil. The agreement stipulates that the weapons must be removed by the end of this year, but does not specify where they should go. Johnston Island is virtually the only place in the world that can handle them, so the plan is that the weapons will be moved by truck, rail and ship, under tight security, from Clausen, Germany, to Johnston Island.

All told, the Johnston Island facility is marked for the incineration of less than 7 per cent of the US stockpile. The current plan calls for the other 93 per cent to be incinerated in the continental United States, at military facilities in Alabama, Arkansas, Colorado, Indiana, Kentucky, Maryland, Oregon, and Utah. Public opposition is already mounting.

Meanwhile, the Soviet chemical weapons stockpile is said to be even larger than that of the United States, and the

Soviet destruction programme is in its infancy. The Soviet president has asked for US help with the Soviet effort to destroy chemical weapons, and collaboration is written into the formal agreement between the two countries. Elisa Harris, an expert on chemical and biological weapons at the Brookings Institution in Washington, says that the Soviet population has even less confidence in its government's ability to do the job than Americans have in the US chemical weapon destruction programme.

She calls the domestic Soviet problem part of a "post-Chernobyl syndrome". The political controversy generated in both countries is likely to make Johnston Island an important model for both sides. US experts have met already with Soviets in Geneva and Moscow to discuss technical issues of chemical weapon destruction, and a Soviet delegation is expected soon to visit a small-scale prototype US facility constructed several years ago at an Army base in Tooele, Utah. As Harris puts it, "the Soviets are clearly capable of destroying their own chemical weapons", but with the domestic political difficulties involved in carrying out such programmes in either country "it will be interesting to see just how much cooperation each side has in mind".

Seth Shulman

CONSERVATION

Tigret spells hope for endangered species

Washington

THE world's first test-tube tiger was put on display at the National Zoo in Washington DC last week. Although the cub was born two months ago, zoo officials have up until now not had the confidence to show it to the

zoo hopes will help to preserve endangered species. Mary Alice is a Siberian tiger, a rare subspecies of tiger of which only 200 remain in the wild. Seven hundred are kept in zoos around the world, which means that captive breeding provides the best chance of saving it from extinction.

The IVF technique is likely to be of particular help because it allows sperm from a valuable male in one country to be used to fertilize eggs from a partner in another country, without all the difficulties of transferring the animals themselves.

Since 1984, the National Zoo, in co-operation with Henry Doorly Zoo in Omaha and the Minnesota Zoo, has been working to adapt IVF techniques originally developed for humans. After IVF was used successfully with domestic cats, the technique was tried on the Siberian tiger. Three test-tube cubs were born in Henry Doorly Zoo on 27 April. A mobile IVF laboratory

developed by the National Zoo and taken to Omaha for the placement of the three fertilized eggs in the surrogate mother tiger will provide artificial breeding technology to other zoos in the future.

Shigeko Segawa

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Test-tube baby — the world's first IVF tiger. public. Two other cubs born at the same time died in their first weeks.

The cub, named Mary Alice, represents a \$2 million dollar investment in *in vitro* fertilization (IVF) technology which the

Jeff Tinsley, Smithsonian Institution

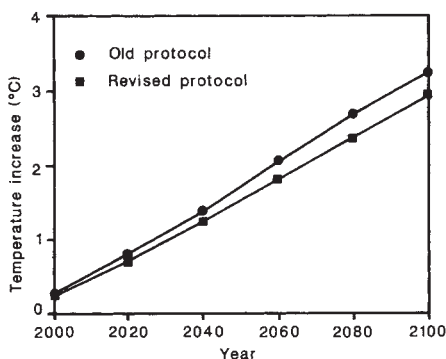
Warming to global agreement

London

UK Environment Secretary Chris Patten hailed last week's Montreal Protocol meeting in London as "a major step forward in environmental diplomacy", after participants agreed to phase out ozone-depleting chemicals more quickly than agreed at Montreal in 1987 and both the Indian and Chinese delegations said they would urge their governments to join the protocol.

The involvement of India and China, which both have the potential to develop large chlorofluorocarbon (CFC) industries, was in doubt until the United States withdrew demands that it should have control over an international fund to help developing countries finance alternatives to CFCs, in proportion to its 25 per cent financial contribution.

Effects of new protocol



New controls on ozone-depleting chemicals outlined in the revised Montreal Protocol will also reduce global warming, as some of the chemicals involved are greenhouse gases. The figure shows the likely effects of the new protocol on increase in global mean temperature from the 1990 value over the next century, carried out for *Nature* by Professor Tom Wigley of the University of East Anglia.

The prediction is from STUGE (Sea level and Temperature change Under the Greenhouse Effect), an interactive microcomputer-based model developed by Wigley and his colleagues Tom Holt and Sarah Raper. STUGE incorporates models used by the Intergovernmental Panel on Climate Change working group I, and Wigley makes the key assumption that a doubling of atmospheric carbon dioxide will increase temperature by 2.5 °C.

Wigley says that his prediction includes some *ad hoc* judgements about the use of the hydrochlorofluorocarbons that are expected to replace CFCs in the short term, and stresses that the model gives a best estimate, around which there may be some margin of error.

The amended Montreal Protocol has only a small effect on global warming, reducing temperature increase between today and 2100 by only about 0.3 °C. But Wigley says: "if we're going to make any progress, it will be the sum total of small effects".

P.A.

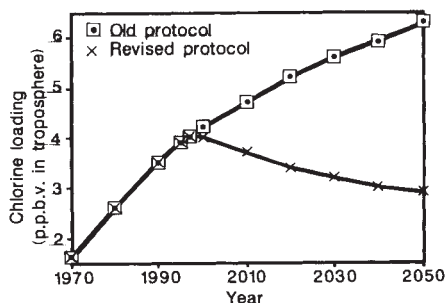
Under the 1987 Montreal Protocol, CFC use was to be halved by 1998, and halon use was to be frozen at 1986 levels in 1992. The new protocol has CFCs phased out altogether by 2000, following a 50 per cent cut by 1995 and an 85 per cent cut by 1997. Halons are also to be eliminated by 2000, except for some essential firefighting uses, following a 50 per cent cut in 1995.

Carbon tetrachloride and methyl chloroform, not covered by the 1987 protocol, are also to be phased out, by 2000 and 2005 respectively. Although these chemicals damage ozone less than CFCs and halons, they are used widely in industry.

Many nations pushed for even quicker progress, and near the conference's end, a 13-nation breakaway group led by Finland announced that its members would end their use of CFCs by 1997, irrespective of the final form of the protocol. US, Japanese and Soviet opposition prevented the conference adopting the 1997 target date, although the target will be reviewed again in 1992. Patten said that the United Kingdom, while not among the 13, would aim to eliminate CFC use by 1997, except for medical uses where alternatives are not yet available.

Scientists at the UK Department of the Environment have produced a preliminary

analysis of the effects of the new protocol on atmospheric chlorine loading (see figure). By 2050, chlorine loading in the troposphere, at 2.9 parts per billion by volume (p.p.b.v.), will be less than half that predicted under the 1987 protocol. Chlorine loading in the stratosphere, and hence effects on ozone depletion, will lag behind this by about five years.



Joe Farman, a member of the UK Stratospheric Ozone Review Group and the discoverer of the Antarctic ozone hole, is worried that even the new controls do not go far enough. He says that the aim for the London meeting should have been to reduce atmospheric chlorine loading back to about 2 p.p.b.v. as quickly as possible. The new protocol will not approach this target until late next century, after concentrations rise to an unprecedented 4 p.p.b.v., a timetable Farman describes as "madness".

Peter Aldhous

BIOMEDICAL RESEARCH

Young researchers go short

Washington

THE hard times being faced by young biomedical researchers in the United States were the major focus of a meeting on 'Support for Biomedical Research' held last week under the sponsorship of the National Academy of Sciences and the Institute of Medicine.

Although the National Institutes of Health (NIH)'s total funding has increased moderately over the past few years, there has been a steady fall in the percentage of successful applications in the 'new and competing' grants category — the grants that launch a young researcher on an independent career. This year as few as 22 per cent of applications are likely to be successful, down from almost double that percentage three years ago.

Many scientists now think that the problem of a shortage of funds was compounded by a decision made in 1985 by James Wyngaarden, then director of NIH, to increase the length of time for which grants run. The decision was made, in part, to provide scientists with greater financial stability and to relieve them of the inordinate amount of time spent renewing grants. But it has had the unintended side effect of intensifying competi-

tion among scientists fighting for their first grants.

Although most scientists agreed that the fundamental problem was a lack of money, David Baltimore, president of Rockefeller University, questioned whether available money was being spent efficiently. He claimed that the Howard Hughes Medical Institute, a nonprofit organization, will win a "disproportionate result" from spending just \$44 million this year to support young scientists.

Industry is continuing to play a large role in biomedical research. In 1979, industry funded 29 per cent of health research and development. Last year, this had risen to 45 per cent. As industry leaders acknowledge their debt to public-sector research, Baltimore suggested that industry could do still more to help by contributing about \$100 million a year to support young researchers and infrastructure development. But Senator Dale Bumpers (Democrat, Arkansas), who serves on the committee that appropriates funds for NIH, said that the federal government "must take a strong lead in basic research" and not leave it to *ad hoc* corporate decisions.

Diane Gershon

Pressurized accounting

London

THE Department of Energy, former Energy Secretary Cecil Parkinson and the now defunct Central Electricity Generating Board (CEGB) were all at fault in the abortive attempt to privatize Britain's nuclear power stations. That is the conclusion of an all-party House of Commons committee which has investigated the spiralling cost estimates for nuclear-generated electricity in the run-up to privatization of the electricity industry last year.

Michael Clark, a Conservative Member of Parliament who chairs the energy select committee, said that these costs more than doubled from CEGB estimates given in 1988. The increases forced the government last November to retain Britain's nuclear stations in public ownership, and to freeze plans to build three more pressurized water reactors (PWRs) besides that now being built at Sizewell B, in Suffolk (see *Nature* 342, 213; 1989).

The report acknowledges that some of the cost increases, for example those due to tightened environmental safety standards, were genuine. But it suggests that the bulk of the increases reflect the fact that the CEGB had never previously provided adequate costings for nuclear generation. The report concludes that "there has been a systematic bias in CEGB costings in favour of nuclear power". Only impending transfer to the private sector forced the CEGB to account for realistically low-risk investment and high returns on capital. After being told for years that nuclear generation was cheaper than other forms of power, it is now "close to the truth to say that nuclear electricity is expensive", Clark said.

The committee censures the Department of Energy and successive governments for failing to obtain realistic nuclear costings from the CEGB. Parkinson is attacked as the minister responsible for privatization, in particular for ignoring the energy committee's advice in July 1988 — which doubted the wisdom of privatizing nuclear power and suggested the government had "glossed over" nuclear costs. But two Conservative members of the committee, Malcolm Moss and Michael Stern, distanced themselves from the attack on Parkinson at the report's launch, because the former Energy Secretary had not been able to give evidence to the committee. An earlier leaked draft of the report had been more forthright in its criticism of Parkinson, saying he had acted like a "dilettante".

The energy committee's report followed close on the heels of a rise in estimates of the cost of completing the Sizewell B PWR caused by contraction of the PWR programme. Costs that would have been shared across the four planned

stations will now be borne by Sizewell B alone. The revised capital cost of Sizewell B, at £2,030 million in 1987 prices, is more than £300 million greater than the original 1987 estimate.

The environmental group Friends of the Earth (FoE), which leaked the figures last week, and opposition Labour energy spokesman Frank Dobson have called for Sizewell B to be cancelled. But John Collier, chairman of Nuclear Electric, the government-owned company that now runs the old CEGB's nuclear stations, said that this would be uneconomic, and dismissed FoE's claim that construction work is running behind schedule. Collier said Sizewell B's costs are not "largely out of control", as FoE had claimed: all the revisions were due to the cancellation of the three further PWRs.

Sam Goddard, director of construction
CHINESE DISSIDENT

Fang gets down to work

London

IN his first public appearance since his surprise release from China, dissident astrophysicist Professor Fang Lizhi made a short statement at the Royal Society in London last Friday. Fang looked forward to "a period of peace and quiet", in which

Associated Press

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Fang Lizhi (left) is greeted by Sir George Porter, president of the Royal Society.

to pursue his research, and declined to answer any political questions.

Fang has now taken up his position at the University of Cambridge's Institute of Astronomy, where he will continue his work in cosmology and large-scale structure of the Universe. Professor Martin Rees, director of the institute, says he is particularly pleased to welcome Fang, who had been extremely effective in promoting academic contacts between China and the West during the early 1980s.

The Royal Society will support Fang's research for up to a year, but Fang may eventually choose to settle in the United States.

Peter Aldhous

and future programmes with Nuclear Electric, said that the cost of electricity from Sizewell B would need to be about 2.5p per kilowatt-hour in order to recover future investment that could be avoided if the project was cancelled. This is similar to the cost of electricity from a new combined-cycle gas turbine plant and less than electricity from a coal-fired station (between 3.3 and 4p per kilowatt-hour). But, if both future and past investment in Sizewell B is used to calculate costs, its electricity is more expensive than either gas- or coal-fired stations.

Clark said he believed that completion of Sizewell B was still justified. Apart from financial considerations, retaining a PWR may be useful in keeping open the option for a later expansion of UK nuclear power. This may prove necessary if 'carbon taxes' are levied on fossil fuels, or enforceable limits on carbon dioxide emissions are introduced, to counter the problem of global warming.

Peter Aldhous

SCIENTIFIC MISCONDUCT

Lawsuit problems

Washington

IN recent months, at least three unexpected lawsuits have introduced a wild card to the investigation of scientific misconduct. In each case, a whistleblower has filed suit — claiming scientific misconduct — under an obscure provision of federal law that allows informants to sue for damages on behalf of the United States and to keep 30 per cent of the damages should they win. Known as a *qui tam* action, such a lawsuit is decided by a jury in federal court, effectively bypassing the scientific misconduct investigation process of the federal science agencies.

The law was designed to encourage whistle-blowing, especially in areas prone to fraud and embezzlement such as defence contracting.

But *qui tam* suits can be quite lucrative in scientific misconduct cases as well. Damages are trebled by law, and if a large National Institutes of Health grant must be repaid times three, a 30 per cent share could amount to many tens of thousand dollars. "Whistle-blowing for fun and profit", is how Crowell and Moring attorney Robert Charrow describes it.

Federal scientific integrity officials and congressional staff are watching the trend closely. "It's a real problem — the purpose of misconduct investigations is not to make anyone rich", says Greg Simon, staff director for the investigations and oversight subcommittee of the House of Representatives Science and Technology Committee. If *qui tam* suits continue, Congress may be forced to try to introduce legislation exempting scientific misconduct from the law, he says.

G. Christopher Anderson

Charles Vest is new president

Boston

AFTER several false starts and a renewed extended search, the Massachusetts Institute of Technology (MIT) has officially chosen its new president. Charles M. Vest, a 48-year old mechanical engineer who is now provost of the University of Michigan, has accepted the job and been formally approved by the MIT Corporation, and is due to begin as MIT's fifteenth president in mid-October this year. At his first news conference at MIT, Vest bemoaned the state of US competitiveness, saying that "large segments of the nation have lost the will to excel". He pledged a "fundamental reassessment of engineering education".

Several leading contenders removed themselves from consideration, and one, biologist Phillip Sharp, declined the job a week after his selection was formally announced. Vest said he is "proud to be a colleague" of someone like Sharp who puts his research above his desire to be an administrator, and brushed aside comments about the long, tumultuous search.

Seth Shulman

JAPANESE SCIENCE

Land of rising publication

Washington

IN the past decade, Japanese researchers have increased their representation in the pages of leading international science journals by over 50 per cent, according to a new survey in *Science Watch*, a newsletter published by the Institute for Scientific Information.

Organic chemistry leads the list of improved Japanese research fields, as measured by journal publication. The five leading chemistry journals recorded an increase in Japanese papers from 8.8 per cent in the first half of the decade to 11.3 per cent in the second half. Applied physics research, especially in the fields of solid state and condensed-matter physics, has been another strong area for Japanese scientists in the 1980s. Representation in top physics journals jumped from 5.1 per cent to 8.5 per cent over the decade.

Biology is the third area in which Japan has shown marked improvement in journal publication. Representation in the five leading biology journals increased from 3.3 per cent to 5.9 per cent in the past decade. But in the two leading multidisciplinary journals, *Science* and *Nature*, Japanese representation has grown only slightly. *Nature* continues to publish more than twice as many Japanese papers as *Science*: the ratio is now 1.8 per cent to 0.8 per cent, as compared to 1.6 per cent to 0.7 per cent a decade ago.

G. Christopher Anderson

Problems of streamlining

Munich

WITH the steamroller of German unification fast approaching, the East German Academy of Sciences last week began the awesome task of stripping away 14,000 of 24,000 jobs in order to create a streamlined core that can be integrated into West German science. Pressure from both the recent currency union and shrinking funds for science forced the East German government to give the academy only until the end of the year to complete its changes. Research institute directors did not learn of this decision until a meeting at the Adlershof research campus in East Berlin on 25 June.

Even the planned reduction will probably not be enough to satisfy the West Germans who will hold the purse-strings for science in a unified Germany. The number of employees who survive the academy cuts is likely to be under 10,000 — but even the renowned Max Planck Gesellschaft of West Germany has just 13,000 employees. That means that an academy with 10,000 employees may still seem excessive when the two German states are unified politically, a step now likely to take place in December. The academy will lose nearly half its budget as the industrial *Kombinate*, which provided support for contract research, disappear in the face of West German competition.

The East German Research Ministry, which is responsible for the academy in the current East German government, has promised to do its best, but is unlikely to be able to take up all the slack as funds disappear. East German Research Minister Frank Terpe said just two weeks ago that he would try to provide a two-year 'moratorium' on the closing of academy institutes to allow time for restructuring and for the influential West German science advisory council (Wissenschaftsrat) to survey academy research projects and recommend changes.

But this timetable is proving too slow for the West German Research Ministry (BMFT) which will take responsibility for the academy in a unified Germany. A BMFT spokesman says that the ministry cannot wait the 18 months requested by the Wissenschaftsrat to see which institutes and groups should survive, but would prefer a "rough assessment" to be completed by December. Though this is "not a perfect solution", he said, "we will try to push in this direction. No matter how much is required for the evaluation", he added, "money will be no obstacle." Wissenschaftsrat will have practical problems carrying out the evaluation so quickly. "It will be hard to get the experts in on short notice," warned Wissenschaftsrat chairman Dieter Simon.

West German Research Minister Heinz

Riesenhuber will meet Terpe, East German Education Minister Hans Joachim Meyer and the heads of West German science organizations this week in order to try to force the pace.

The new East German scientific leaders have additional worries. Benno Parthier, just appointed director of an academy research institute in Halle and president-elect of the Akademie Leopoldina, said that such a quick change would not leave time for a "differentiated look" at what East German science could do.

Horst Klinkmann, a medical researcher in Rostock who last week became the first democratically elected president of the academy, said that if there is not enough time to adapt, it will leave a "scientific vacuum" in East Germany that could destroy the economy.

BMFT wants different institutes to receive different treatment. Some would be shut down or sold to industry — these would be easy to identify, according to a BMFT spokesman. Others will be maintained, after excess has been pared away, as institutes for applied research under the Fraunhofer Gesellschaft or perhaps even as part of the Large Research Establishments (*Grossforschungseinrichtungen*). There will be plenty of painful choices, especially within institutes that have to be reduced in size, "I have to consider the existence of the institute first, before the jobs of individual employees," said Parthier, who is giving up his summer holiday to begin the task. "But I have known these people for many years, and it is not their fault that they are in this position."

East Germans who had hoped for salvation through the Max Planck Gesellschaft (MPG) will be sorely disappointed by the announcement at the MPG annual meeting in Lübeck on 20 June that East German institutes would not be absorbed. Incoming MPG president Hans Zacher said the dialectical approach to science in East Germany had "turned on its head" the successful West German approach (which originated in the nineteenth century under the Reich) allowing freedom of research with generous state and industry support. "Trying to unify the two systems could destroy them both", he warned. "There is a lot of useful research in East Germany. . . . Our problem is how to integrate it into a system based on freedom without changing ourselves." Simon, who is also director of a Max Planck Institute, considers such fears unreasonable. "We will not be 'infected' by the East German mentality, as some are supposing", he added. "Instead", he said, "we have to develop a 'we' mentality" together with the East Germans.

Steven Dickman

Investigators investigated

Washington

A year after it was first created, the National Institutes of Health's Office of Scientific Integrity (OSI) is still getting an on-the-job lesson in the difficult art of investigating scientific misconduct. Faced with a growing case load (nearly 80 at the last count), congressional and media scrutiny and criticism from universities and scientists who have passed through its doors, OSI is under pressure to prove in its second year that it can deal effectively with misconduct without stifling research.

The first step it will take is to publish, in the coming weeks, a set of formal procedures describing how OSI works. Included will be rules that will give those found guilty of misconduct a chance to negotiate their own sanctions and those found innocent a greater opportunity to clear their names. Congress is also considering taking matters into its own hands. A draft bill now being circulated by the subcommittee on investigations and oversight of the House of Representatives Science Space and Technology Committee would set new guidelines to assure fairness in university misconduct investigations and increase the protection of whistle-blowers.

Legal rights?

The lack of any published procedures has fuelled the principal complaint against OSI that it fails to accord basic legal rights to people accused of misconduct. And, because no one knows what action is being taken, OSI appears to take far too long to complete an investigation — the inquiry into the case of the *Cell* paper written by David Baltimore and others has now been running for over two years.

Another celebrated case, that of AIDS researcher Robert Gallo, raised new criticisms when OSI decided to appoint a National Academy of Sciences panel to oversee its investigation. If an oversight panel was needed in this case, why not in others? "It would appear that the entire *ad hoc* 'process' has been devised with at least one purpose in mind — protecting NIH from congressional criticism", wrote former Health and Human Service (HHS) deputy counsel Robert Charrow in last month's *Journal of NIH Research*.

The most severe criticism of the NIH investigatory process comes from those who have suffered at its hands. "Potentially career-destroying investigations should not be carried out the way mine was", says David Bridges, a Purdue University vision researcher who was found guilty of plagiarism by an NIH investigation last year (see *Nature* 340, 173; 1989). In the three years since allegations were first made in his case, Bridges says he has spent uncounted hours and well over

\$20,000 of his own money on his defence. He still protests his innocence and is the only researcher to have gone through every appeal process up to and including a just-completed four-week debarment hearing.

Bridges must now wait until the end of the year to learn if, as a result of the hearing, the Department of Health and Human Services (HHS) will reverse the earlier NIH decision or concur and ban him from receiving federal grants. Until the debarment hearing earlier this month, Bridges claims that neither he nor his lawyers had been allowed to cross-examine witnesses or take testimony on the record.

Under the current NIH rules, a researcher "can be debarred without even getting the chance to testify", complains Bridges' lawyer, Houston attorney Leslie Ribnik. Debarment hearings are granted at the discretion of HHS. There are no laws that compel NIH to call the accused researcher to give evidence during earlier investigations, Ribnik claims.

University regulation

Although OSI has operated without making its own procedures public, it has laid out a set of rules for how universities receiving HHS money should conduct their misconduct hearings.

Once alerted to the possibility of misconduct, the university is expected to put together a panel to review the charges in no more than 60 days. If the panel finds cause for a formal investigation, the university is to conduct an investigation within 120 days. At the investigation stage, the university must inform NIH and submit a report on the outcome to OSI. If it is dissatisfied it may then carry out its own investigation.

Although institutional guidelines have been in print for over a year, there are still doubts over whether they are fair, in the sense of whether they grant the accused adequate rights of "due process".

One problem is that at no time is an accused researcher guaranteed the right to see the evidence or hear the charges against him or her. "You can spend a lot of money trying to respond to unnamed people and uncharacterized charges. Meanwhile, your funding can be halted and your grant application can be denied", says Barbara Mishkin, an attorney with the Washington law firm of Hogan and Hartson.

But Suzanne Hadley, OSI's acting director for most of its first year, says that granting full access to evidence and written transcripts has become OSI policy, even if they are nowhere published. Another unwritten OSI policy is to spell out the charges, although OSI sometimes draws the line at revealing the informant.

"We try to depersonalize things — to treat it as a federal office making the accusations. Too often the whistle blowers feel that they're carrying the whole fight on their own shoulders", she says.

The draft congressional bill provides for even greater whistle-blower protection but its authors are wary of going too far. Greg Simon, staff director of the oversight and investigations subcommittee, is concerned that overprotection of informants could create "a new tenure track". If whistle-blowers are made sacred, universities may find themselves unable to dismiss them for legitimate reasons years later, he says. Meanwhile, both OSI and NIH as a whole are considering sanctions against those who make false accusations.

Due process

Simon's bill proposes more emphasis on due process, at least at the university level. For researchers under university investigation, the bill lays out a series of rights, including the right to an attorney, to call and cross-examine witnesses, to present evidence and to file a statement. In return, for universities that accept the guidelines, the bill offers protection against lawsuits by accused researchers.

Full due process within NIH investigations is a stickier issue. Hadley argues that it is inappropriate for OSI. "What we do is not a trial", she says. But others point out that because OSI reports directly to the Office of Scientific Integrity Review, the overseeing body within HHS that recommends sanctions, the combination has the effective force of a judge and jury. "A judicial procedure has due process, an investigation doesn't. 'Which is OSI?' asks Charrow.

Inside NIH, criticism of the investigation process has been fanned by messy cases that last years and draw congressional charges of bias or whitewash. "I do have the feeling that these cases should be investigated more diligently and promptly. When misconduct investigations drag on, the public thinks there are more and more of them", says Edward Rall, director of intramural research.

Rall believes that much of the problem lies in the initial investigation by the university. "I'm not sure that all the universities understand that they can't cover up. When they close ranks, they only postpone the moment of truth."

Although NIH plans at present to retain the policy of letting the university do the first inquiry, it is fine-tuning other OSI procedures. Under consideration are 60- or 120-day deadlines — similar to the guidelines for university investigation — on the various stages of the OSI process. But it may be too soon to look for clockwork precision. Says Hadley, "the length of the investigations is one concern — but a lack of balance and objectivity is another."

G. Christopher Anderson

History of Australian prehistory

SIR—A recent News item by Tania Ewing (*Nature* 345, 9; 1990) contains a number of inaccuracies and misconceptions about the department of prehistory in this school, apparently based on allegations made by Dr Johan Kamminga. One is that there has been a 'Cambridge cabal' ensuring that Cambridge graduates dominate the staff appointments and that British graduates get the more senior posts. The facts are that since its establishment in 1961 there have been only seven tenured appointments in the department of prehistory. When the first two tenured appointments were made in the early 1960s, there were no PhD graduates in prehistory from any Australian university. Any appointees would have required overseas qualifications. At the time of their appointments, tenured staff held a total of 16 degrees from courses of university studies. Of the 16 degrees, only four were from Cambridge. Five were from Sydney. In 1989, the nine academic staff (tenured and non-tenured) held a total of 21 degrees, three of which were from Cambridge. Five were from Sydney.

Neither is Cambridge dominant as a source of students. Of the 32 students who have completed PhDs in the department, only three took their first degree at Cambridge. Four were from Sydney from a total of 12 from Australian universities.

The assertion that Cambridge dominates is not supported by the facts. One could claim a Sydney network with more justification, but such a claim would be equally untrue. The school, including the department of prehistory, seeks to make the best possible appointment from applicants on the basis of merit, not place of origin.

A major misconception in the article leads to the implication that, following the 1978 review of the school, the department of prehistory is at fault in not shifting its interest towards South-East and East Asia. This was not a general requirement throughout the school. Neither was recruitment of students from these areas a requirement, and indeed has never been a deliberate policy. The school takes students, and awards scholarships, on a competitive basis from all over the world, without any regional preferences. The department of prehistory recruits students interested in working in its field areas, Australia and Melanesia, regardless of the place of origin of the students.

The assertions that electoral committees have been "stacked", that Australian graduates get junior posts while "British

graduates get the more senior positions" or that the department of prehistory in this school is a "Cambridge academic colony" are quite simply untrue.

R. GERARD WARD

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SIR—Before your readers conclude that the prehistory department at the Australian National University is a 'Cambridge circus' and especially before there is a rush of Cambridge graduates looking for certain tenured employment in Canberra, I hope you will allow an all-Australian member of the department to correct Tania Ewing's report.

No member of the department has seen Dr Johan Kamminga's allegations so it is difficult to judge whether they are all as inaccurate as the ones he makes public.

In its 21-year history, this research department has had only seven tenured academic staff. Four had Cambridge undergraduate training but two of these obtained Australian PhDs as the basis of their appointment here.

Jack Golson and John Mulvaney were recruited from Auckland and Melbourne respectively to found Australia's first prehistory department in 1969, so by definition their training was non-Australian. It is not surprising that they both received

it at Cambridge, then, as now, one of the most distinguished centres of teaching and research in prehistory.

Golson and Mulvaney's ethical and research standards have been major reasons behind the dramatic expansion of prehistory in this region and why the indigenous people of the area have become so interested in it.

It is therefore outrageous for Kamminga to claim that these scholars, two of the world's most respected prehistorians, 'fiddled appointments' from the time they arrived in Canberra.

The next tenured post in the department was not filled until 1972, but your readers can make their own assessments as to whether the subsequent tenured staff — Rhys Jones, Jim Allen, Doug Yen, Matthew Spriggs and myself — had to be 'fiddled' or were appointed because of our publications and research skills.

It is interesting that Kamminga's degrees, like my own, are from the department of anthropology in the University of Sydney. Of the seven tenured prehistorians at our *alma mater* over the past 29 years, all but two have had at least one Cambridge degree. I am certain though that, like us, they simply make the best appointments on the increasingly rare occasions when there is a vacancy to fill.

ALAN THORNE

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Ungodly thoughts

SIR—Am I alone in being surprised at the number of debates, in the pages of *Nature*, on religion and the existence of god? Most recently this was apparent in the review of Sir John Eccles' new book (*Evolution of the Brain: Creation of the Self: Nature* 344, 117; 1990), and the subsequent reply from god (as revealed to Ralph Estling, 344, 582; 1990). Although, as in this example, references to god in *Nature* are usually frivolous, this is not always the case (see, for example, 337, 498; 1989), and the ways in which religion affects the world at large are certainly not a laughing matter (as I am sure Mr Salman Rushdie would testify). Yet *Nature* seems to be according respectability to religious beliefs.

As scientists, should we not be protesting at the way religions permeate society and intrude into our lives, whether or not we want them to? Or, if not protesting, at least making our views known? In a straw poll that I conducted in this medical school, only eight of the 63 people I questioned had any form of religious belief. I suspect that this low incidence of religious beliefs is true of scientists in general, but it would be nice to know for sure.

I would like to suggest that *Nature* should conduct an opinion poll on the

religious beliefs of its readers. If my small sample was typical, such a poll could indicate the strength of the atheist lobby (the silent majority?) in science. What would this achieve? Probably nothing, but who knows? Governments might resolve to bring to a halt the religious indoctrination of children. If children were informed on how many different religions there are, and on the fact that if any one of them happened to be right, all the others would be wrong, they might without much difficulty deduce the likelihood that all religions are wrong. Religious programmes on radio and television could come with a government health warning. People might even begin to realize that what happens to our world depends on us.

Nature would be performing a valuable public service by such a survey. The international readership of the journal would enable us to ascertain whether the prevailing religion of different countries influenced the extent of rejection of religious belief by scientists. Whatever the outcome, it would be good to know how many of us think that man created god (in his own image, of course).

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Letters submitted for Correspondence should be typed, double-spaced, on one side of the paper only.

Carbon dioxide will be harder

Last week's successful review conference of the Montreal Protocol on ozone would have served an even better purpose by spending more time on verification and compliance.

LONDON had hardly said goodbye to last week's gathering of the parties to the Montreal Protocol on ozone than it was filled with the delegations of the members of the North Atlantic Treaty Organizations, who will be discussing (among other things) the prospects for further arms control measures in Europe. There seems little doubt that the people at the earlier gathering are a more trusting lot than those at the second. What does either group have to learn from the other?

For decades, it has been taken for granted that there can be no arms control without verification. The assumption has always been that the stakes are too high to take the other fellow's word on trust. This is one of the reasons (there were several others) why, in 1979, the then US President Jimmy Carter did not send the SALT II treaty (on strategic arms) to the US Senate for ratification: as negotiated, it allowed only for 'national means' of verification, which was a euphemism for the use of surveillance satellites. By contrast, the treaty on nuclear missiles of intermediate range, signed at the end of 1988, and the START treaty now being negotiated, include provisions for the direct inspection of disarmed missiles, sometimes on unprovoked demand.

The Montreal Protocol does not yet have teeth like that. Even in its newly tightened form (see page 6), the convention is not especially specific about the baseline data, for 1986 and 1989, against which future reductions of chlorofluorocarbons (CFCs), and variants on that theme, are to be assessed. The secretariat requires of the governments that are members of the convention that they supply details of production and consumption of the various materials interacting with ozone in the stratosphere. And where will those data come from? The only possible sources are the companies that manufacture them, and which must be supposed to have a vested interest in inflating their first estimates so as, later, to be less hurt by the proportionate reductions to which they have agreed.

At least on past form, the arms controllers would not so easily have taken what their fellow signatories had to say about their own dispositions as synonymous with the truth. In the absence of 'national means of verification', there would have been calls for lists of the manufacturing plants at which offending operations were

being carried on, as well as for opportunities for inspecting them. For what it is worth, the signatories of the Montreal Protocol might reasonably have bound themselves to provide a list of the plants manufacturing CFCs and the other substances now controlled, if only so as to be better able to tell what to make of newly constructed plants if they should make their appearance in the years ahead.

It is also surprising that the Montreal Protocol is as innocent as it seems of formal arrangements for the systematic monitoring of the now-controlled materials. The ostensible objective of the enterprise is to regulate the concentration of these chemicals in the stratosphere, for which purpose the tropospheric concentration is a legitimate proxy. But the measurements by which the supposed increasing concentration of, for example, CFCs has been verified have mostly been carried out with air samples collected from oceanic islands.

That makes sense if the objective is to detect trends unencumbered by locally generated noise, the nearness of a chemical plant for example. Now that the convention has been made more stringent and its scope extended, its members cannot be expected to continue to rely on whatever measurements of this kind independent investigators choose to carry out, but it is plainly of more than passing interest to them to know something about sources of regional or even local noise that may betoken the presence of unsuspected chemical factories.

These issues have been given close attention over the years in negotiations to control the manufacture of chemical weapons, mostly at Geneva (in the building in which the United Nations Environmental Programme has its European office). Not so long ago, the Finnish authorities explored the use of civil aircraft equipped with air samplers and gas chromatography as a means of telling what kinds of chemical plants lie beneath civil airline routes. Fitting the exhaust stacks of all chemical factories with sealed automatic samplers would be more effective, but would encounter the objection raised, during the test-ban negotiations in 1978, that remotely operated seismometers would provide much more extensive data than were required for the working of the treaty. Even in the cause of the Montreal Protocol, not every chemical manufacturer would welcome others knowing what

flows up his chimney stacks.

Yet another lacuna in the new arrangements is financial, and concerns the procedure for recompensing developing countries for their willingness not to make CFCs, but only the substitutes for them, and to lag no more than ten years behind the rest in full compliance. It is, of course, remarkable that so many rich countries have agreed to contribute to what is called the multilateral fund to compensate developing countries for their self-denial. But it will be a headache for the lawyers and the accountants. The preamble to last week's conference document records the need to avoid 'double-counting'. But only trusting folk like the ozone people would have let things get this far without calling in teams of lawyers to draft their resolutions.

That is as it should be. It would have been absurd to have invested the ozone negotiation with all the niceties of the arguments there have been in the avoidance of nuclear war. That does not imply that the ozone problem is not serious, but that it is a less serious problem than the threat of nuclear war. And, as the signatories of the Montreal Protocol openly confess, the last thing on their agenda was to frighten off non-signatories by too elaborate a system of control. Fair play, the strategy has worked. It is a victory that China and India may now join.

So why rehearse the gaps in the convention that will send the world's arms controllers into shivers? Because the convention is more than a means of reducing the incidence of skin cancer, it is a model for the convention there will soon have to be on the control of the emission of greenhouse gases (among which the CFCs are incidentally important in their own right). And there the stakes will be much higher, perhaps even comparable with the threat of nuclear war. People will be less willing than over ozone to trust others to tell the truth, from the fear that they may be lying.

That is why a few arms controllers might usefully get together, in the wake of last week's successful conference, to advise the ozone people on the ways in which their protocol might have been made not just a declaration of good intentions, but watertight as well. The exercise would of course be unnecessary and thus academic, but a version of it will be needed when work begins on carbon dioxide.

John Maddox

Good things come in threes

Charles Bailyn

THE high density of globular star clusters is believed to be conducive to pulsar formation. But although recent years have indeed seen a stream of pulsar discoveries in these systems (19 are now known, whereas none were three years ago), the observations continue to bring forward as many questions as they answer. The discovery by Anderson *et al.* of the second and third pulsars in the very dense cluster M15 (Fig. 1), reported on page 42 of this issue¹, is especially significant, both for the innovative method used to detect them and for the interesting properties of the two pulsars and of the cluster itself.

Pulsars are rapidly magnetized neutron stars. They emit beams of electromagnetic radiation from the magnetic poles, which are observed on Earth as pulses of radio emission as the beam sweeps across once with each rotation. The pulsars in globular clusters belong to a subclass often referred to as 'millisecond' pulsars, although in fact their observed pulse periods range from a few milliseconds to a few tenths of a second. The search for pulsars usually involves taking a Fourier transform of a long time series of radio measurements to look for a periodic signal amidst the background. This search must be made for different values of the dispersion measure — the amount of ionized gas between the observer and the pulsar — because the speed of light for a given frequency depends on the column density of this intervening plasma. Without a correction for this effect, the pulse arrival time will vary with frequency, resulting in an undetectable 'dispersed' pulse. But there is little, if any, gas within globular clusters, so the dispersion measure is the same for all pulsars in a given cluster. Anderson *et al.* have taken advantage of the previous discovery² of a pulsar in M15, from which was determined the dispersion measure towards the cluster, to greatly increase the sensitivity of their search, which has resulted in the discovery of two fainter pulsars.

The improved sensitivity comes about in two ways. First, long series of data can be analysed with much less computing effort, as only one value of the dispersion measure need be considered. Second, a strong 'selection effect' that normally mitigates against finding pulsars in binary star systems with short orbital periods can be at least partially removed. The selection effect arises because the observed pulse period varies, and the signal in the Fourier transform is consequently reduced, as the pulsar in a binary system is accelerated relative to the Earth by its orbital motion. Anderson *et al.* have used the improved computational efficiency

made possible by knowing the dispersion measure to search for the variable pulse periods that would be created by a range of constant accelerations. This procedure resulted in the discovery of the third pulsar, PSR2127+11C, which could not otherwise have been detected.

Indeed, it is the short orbital period (about eight hours) of PSR2127+11C that

pulsars are slowing down. This is not thought to be an intrinsic property of the pulsar, but instead a result of the strong gravitational acceleration caused by other stars in the cluster. Thus measurements of pulsar timing can probe the cluster's gravitational potential. The locations of the pulsars within the cluster reveal the operation of dynamical processes: pulsars A and B, for which positional information is available, are within a few arcseconds of the cluster centre, indicating that these relatively massive systems have fallen there as equipartition of energy has

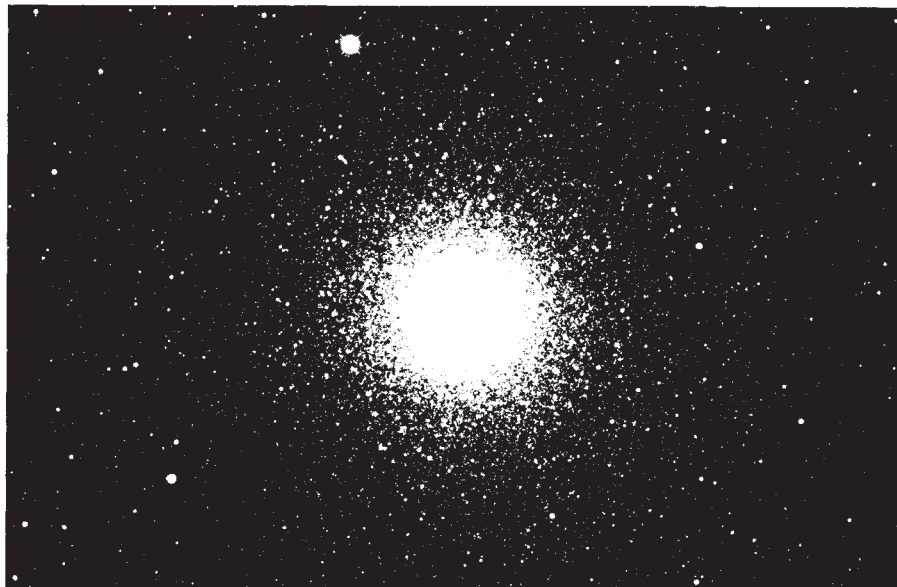


FIG. 1 An optical image of M15 taken at the Kitt Peak National Observatory. The two pulsars (A and B) for which accurate positions are known are located at the centre of the bright region.

makes its discovery particularly exciting. Its orbital parameters are remarkably similar to those of PSR1913+16, which has been used for a sensitive test of general relativity³. The exquisite accuracy possible for timing measurements on short-period pulsars means that it is feasible to look for various relativistic effects, including the decrease in period of the orbit through emission of gravitational radiation. These effects should be observable in PSR2127+11C within a few years.

M15 is itself an interesting cluster, being the best-studied member of a class of clusters that have strong cusps in their central density (Fig. 2). These cusps are a signature of an advanced stage of cluster evolution. The high central density should give rise to a large number of stellar collisions and near misses, which are thought to result in the formation of accreting neutron stars in binary systems — the precursors, according to the standard scenario, of millisecond pulsars. Thus M15 should be a fertile breeding ground for pulsars, as the new observations seem to confirm.

The three pulsars in M15 provide probes of the cluster's dynamics. The first of them² shows an unprecedented increase in spin rate with time — all other

become established through interactions with other cluster stars. Finally, the fact that two of the three pulsars in M15 are not binaries is in itself revealing in terms of their dynamical interactions with other stars. Although millisecond pulsars are thought to be born in binary systems, a recent study⁴ of the dynamics of M15 shows that encounters with a third star are likely to lead to the 'ionization' of a binary pulsar (that is, the loss of its companion).

But the stories told by pulsars about their parent clusters are not limited to the clusters' dynamics — they also reveal problems with generally accepted theories of stellar evolution. The basic difficulty is that there are so many globular-cluster pulsars. As distances to globular clusters are large, only the very brightest pulsars will be observable; thus the pulsars observed probably represent only a small fraction of the total population. Recent extrapolations^{5,6} suggest that there may be as many as ten thousand pulsars in galactic globular clusters. Neutron stars are created in supernova explosions of stars with ten or more times the mass of the Sun, so this large number of pulsars requires a large primordial cluster population of massive stars. In M15 and several other clusters, however, studies⁷ of the

QUANTUM MECHANICS

No need for nonlinearity?

Richard Thompson

remaining unevolved stars (all less massive than the Sun) show that the number of stars drops off very sharply with increasing mass. Thus the function that describes the variation with mass of the frequency of occurrence of stars must be more complicated than the simple power law that is usually assumed.

This requirement for a large number of neutron stars also poses problems for the stability of the clusters as a whole, because the mass loss during the evolution of a large population of massive stars has been shown to result in complete disruption of

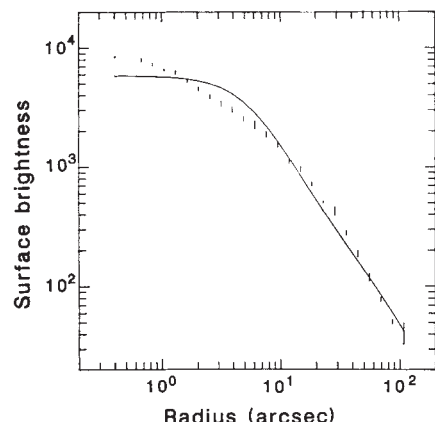


FIG. 2 Radial density profile of M15 (from ref. 9). At small radii, the data (short horizontal lines) considerably exceed the predictions of the King model¹⁰ (solid line), which fits most globular clusters.

the cluster⁸. Furthermore, neutron stars in binaries are spun up to 'millisecond' periods by accreting material with high angular momentum from their companions. Such accreting neutron stars are indeed observed in globular clusters as strong X-ray sources, but estimates of the birth-rates of these systems are orders of magnitude too small to account for the inferred number of pulsars^{5,6}.

Now that we know where and how to look for millisecond pulsars, we can expect the stream of discoveries to become a torrent. There should soon be many more opportunities to exploit the unique tests of gravitational theory provided by pulsars, as well as to learn more about the evolution of stars and star clusters. □

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1. Anderson, S.B., Gorham, P.W., Kulkarni, S.R., Prince, T.A. & Wolszczan, A. *Nature* **346**, 42–44 (1990).
2. Wolszczan, A. *et al.* *Nature* **337**, 531–533 (1989).
3. Weisberg, J.M. & Taylor, J.H. *Phys. Rev. Lett.* **52**, 1348–1350 (1984).
4. Rappaport, S., Putney, A. & Verbunt, F. *Astrophys. J.* **345**, 210–221 (1989).
5. Bailyn, C.D. & Grindlay, J.E. *Astrophys. J.* **353**, 159–167 (1990).
6. Kulkarni, S., Narayan, R. & Romani, R. *Astrophys. J.* (in the press).
7. McClure, R.D. *et al.* *Astrophys. J.* **307**, L49–L53 (1986).
8. Chernoff, D.F. & Weinberg, M.D. *Astrophys. J.* **351**, 121–156 (1990).
9. Luger, P.M., Cohn, H., Grindlay, J.E., Bailyn, C.D. & Hertz, P.L. *Astrophys. J.* **320**, 482–492 (1987).
10. King, I.R. *Astr. J.* **71**, 64–75 (1966).

CLASSICAL oscillatory motion, such as that of a simple pendulum, can become nonlinear at large amplitudes, whereby a dependence on amplitude starts to creep into the resonant frequency. Last year Weinberg¹ proposed that similar nonlinear corrections might be necessary to the wave equations of quantum mechanics: this idea was tested subsequently to high accuracy in a study of trapped beryllium ions by Bollinger *et al.*². Chupp and Hoare³ have now reported a similar test from a different kind of experiment based on spectroscopy of the ²¹Ne nucleus. They find that any nonlinear corrections to the usual linear quantum-mechanical formulation must be tiny: smaller than 1.6×10^{-26} of the binding energy per nucleon in ²¹Ne atoms.

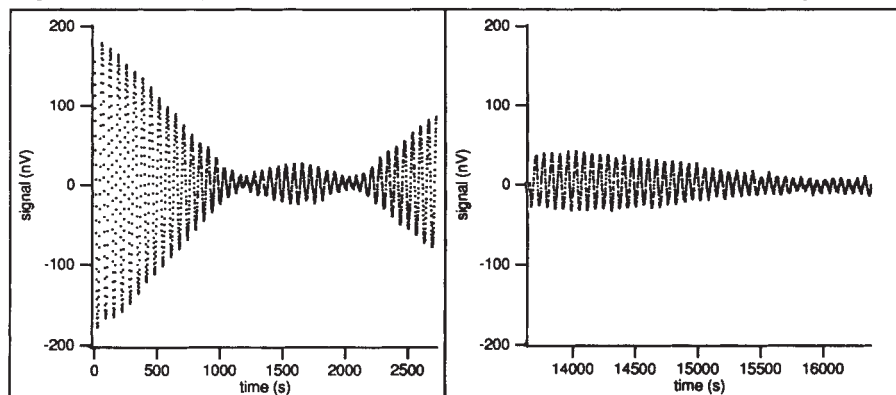
Although quantum mechanics has no difficulty in providing a description of nonlinear effects, Weinberg^{1,4} revived the question of whether the usual linear form of quantum mechanics is complete. He proposed a class of possible corrections to the usual hamiltonian function, which is linear in the product of ψ and ψ^* (the wavefunction and its complex conjugate). These corrections are nonlinear in the product of ψ and ψ^* and can be cast in such a form that the hamiltonian still describes separated systems correctly. The consequences of such corrections can be calculated and tested in the laboratory; but there is as yet no experimental evidence that they are necessary. One particular consequence would be that the principle of linear superposition of wavefunctions would no longer hold. It is this aspect that the experiments set out to test.

I have discussed the Be⁺ experiment previously⁵. It involved the investigations of an essentially two-level system within the hyperfine structure of the ground state of ⁹Be⁺. A cloud of ions was held in an ion trap and cooled by collisions with laser-

cooled Mg⁺ ions held in the same trap. The ions were prepared in a superposition of the two levels by a microwave pulse and the precession frequency of the nuclear spin in an external magnetic field was measured to very high precision. Nonlinear corrections to quantum mechanics would show up as a dependence of the precession frequency on the degree of excitation of the ions — the frequency should change as the amplitude of the excited state in the superposition state is changed. No nonlinearities were detected at the level of 4 parts in 10^{27} of the binding energy per nucleon of the ⁹Be nucleus.

Chupp and Hoare³ have chosen to study the ground state of the ²¹Ne atom, which has a nuclear spin of 3/2. The atoms are placed in a weak (about 3 gauss), uniform magnetic field B , which splits the ground state into four levels (labelled by the quantum number M_I), such that the three transition frequencies between pairs of adjacent levels are all about 995 Hz. These three frequencies are monitored as a function of the degree of alignment ('tensor polarization') of the system: a measure of the degree to which the system is in the outer ($M_I = \pm 3/2$) states rather than the inner ($M_I = \pm 1/2$) states. Nonlinearities of the simplest form to satisfy the conditions mentioned earlier will cause these frequencies to vary in a particular manner.

Assuming that the nonlinearity is characterized by a small parameter ϵ (with dimensions of energy), each of the four M_I levels is shifted by an amount proportional to ϵ and dependent on the exact state that the system is in. If ω_1 is the transition frequency between the $M_I = 3/2$ and $1/2$ levels, and ω_3 is the transition frequency between the $M_I = -3/2$ and $-1/2$ levels, it turns out that the difference frequency $\omega_1 - \omega_3$ is equal to $(2+T_1)\epsilon/\hbar$, where T_1 is the alignment of



Coherent free precession of ²¹Ne over 4.5 h. The signals show beats due to the tiny differences between the three transition frequencies for the four-level system. The traces can be fitted to theoretical curves to extract the three frequencies and their damping coefficients; the degree of alignment can be extracted from the relative amplitudes of the frequencies.

the system. Thus if the system is prepared in superposition states with varying amounts of alignment, this difference frequency should change if nonlinearities are present. The technique, developed from earlier experiments that searched for violations of Local Lorentz Invariance⁶, uses a cell containing both ^{21}Ne and ^3He . The nuclear spin of ^3He precesses in the magnetic field, but shows no nonlinear effects as it has a spin of only $1/2$. The precession frequency is about 9,600 Hz, which is divided electronically by 9.649 to give a reference frequency about $1/60$ Hz away from the ^{21}Ne precession frequency. The beat signal between the two frequencies can be followed for a period of 2,700 s before the He signal decays. The Ne signal can, however, be measured over a period of 4.5 h if the helium atoms are re-excited periodically. The neon atoms are initially polarized (put into specific spin states) via spin-exchange collisions with Rb atoms excited by laser irradiation. Once the polarization is established, the cell is cooled to freeze out the Rb vapour, and a pulse of resonant oscillating magnetic field tips the neon spins by 20° . Their free precession is then monitored with a pickup coil. A critical feature of the experiment is that any small variations in B do not influence the results, as the helium atoms providing the reference frequency sample the same field as the neon atoms. The important difference frequency is then relatively insensitive to the field fluctuations.

A typical trace of the beat signal is shown in the figure. From these traces, $\omega_1 - \omega_3$ can be found as a function of T_1 , giving a limit for ϵ/h of $12 \pm 19 \mu\text{Hz}$. Comparing this with the binding energy per nucleon of the ^{21}Ne nucleus gives a fractional nonlinearity of less than 1.6×10^{-26} , similar to the Be ion-trapping experiment.

What does all this signify? The Weinberg model has been designed to incorporate nonlinearities in as general a manner as possible, but it is of course possible that a different model is needed. It is also possible that nonlinearities are present but only at an even smaller level; in that case their detection may not be feasible, as these experiments are already extremely sensitive because of the very long interaction times involved. But it seems most likely that a linear quantum-mechanical description of matter is sufficient and that nonlinear corrections are not necessary. $\square$

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- Weinberg, S. *Phys. Rev. Lett.* **62**, 485–488 (1989).
- Bollinger, J. J. et al. *Phys. Rev. Lett.* **63**, 1031–1034 (1989).
- Chupp, T. E. & Hoare, R. J. *Phys. Rev. Lett.* **64**, 2261–2264 (1990).
- Weinberg, S. *Ann. Phys.* **194**, 336 (1989).
- Thompson, R. *Nature* **341**, 571–572 (1989).
- Chupp, T. E. et al. *Phys. Rev. Lett.* **63**, 1541–1545 (1989).

Two dietary developments

Michael A. Taylor

KIPLING¹ traced the origin of the baleen whales to one which unwisely ate a “shipwrecked Mariner of infinite-resource-and-sagacity”, who cut up his raft into a small square grating and rammed it down the animal’s throat. Today’s hypotheses for the origin of major vertebrate groups are tested rather more rigorously by fossil evidence and internal logic. Two recent and apparently disparate examples show how illuminating that approach can be

them break or gnaw through the shell, but the deer simply swallow the seeds whole for digestion and softening by rumen microorganisms, before rechewing.

In Bodmer’s view², the ancestral ruminant stomach originally evolved to cope with such tough fruits, and then became used increasingly to digest other fibrous plant parts such as leaves. Interestingly, brocket deer show this dual use, eating more nonfruit material when stranded on

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Ichthyosaurs — these creatures may have evolved from shallow-water predators.

where a group’s key adaptation has changed its function, or when the original shape of members of a group has been transformed. In one report², Bodmer argues that the stomach of ruminant mammals such as cattle and sheep, now used to digest grass and fibrous plant material, evolved to deal with tough fruits. In the other³, Massare and Callaway show that ichthyosaurs, the marine reptiles famous for resembling sharks and dolphins even down to the deep double-lobed tailfin, did not even have tail fins to start with, but that such tails became advantageous with a change in feeding patterns.

The stomach of ruminants contains a compartment, the rumen, in which plant food is fermented by symbiotic microorganisms in between regurgitation and further chewing (as the cud). Otherwise indigestible fibre can thus be broken down into assimilable nutrients: this has enabled ruminants to become today’s dominant large herbivores feeding on fibrous grass or browse. But fermentation tends to waste free nutrients already present in high-quality food such as fruits, so that the ruminant stomach would seem to be a handicap to frugivores. Fossil evidence indicating that the first ruminants fed on fruits in Eocene tropical forests (50–42 million years ago) seems, therefore, strange.

Today’s closest equivalents to the original ruminants are the red and grey brocket deer (*Mazama americana* and *M. gouazoubira* respectively), small Amazonian forest frugivores. They feed especially on palm fruit, the seeds of which are highly nutritious but heavily protected against attack. Most mammals that eat

temporary islands during floods. As the Eocene forests were very similar to Amazonia today, this is a tempting model for the transition.

The diversification of ruminants during the Tertiary coincides with the decline of nonruminant ungulates, not because they were inherently superior, but probably because climatic and vegetational changes, such as the development of grasslands, favoured their ability to process small amounts of food efficiently (the non-ruminants’ form of intestinal fermentation requires more food and a greater throughput of it)⁴. But nonruminants can be superior under different conditions. So the new hypothesis² has the merit of invoking a specific qualitative advantage of rumen fermentation: the fruits can be fermented and then returned to the mouth for further chewing in a manner which would be impossible for otherwise efficient hindgut fermenters, unless they consume their own faeces: hardly practical for a large, mobile mammal.

In the second example, Massare and Callaway³ use new data on ichthyosaurs from the Triassic period (248–213 million years ago) to conclude that ichthyosaurs are most probably members of the Diapsida, a large group of reptiles characterized by the presence (in most) of two pairs of openings in the sides of the skull behind the eyes, for jaw muscle attachments. If so, ichthyosaurs must have lost the lower pair of openings. Their origins lie among the primitive lepidosauromorphs (the diapsid group including tuataras, lizards and snakes), and the apparent discrepancies — such as the lack of a breastbone, present in primitive lepidosauromorphs

— seem to be related to their transformation into aquatic reptiles.

A near-monopoly of marine reptiles in the Mesozoic (250–65 million years ago) was thus enjoyed by the Diapsida, which apparently include the placodonts⁵ and nothosaurs (and by implication plesiosaurs)^{6,7}, as well as the sea-crocodiles and mosasaurs (giant marine lizards). Was this just luck, or was the diapsid skull particularly effective for aquatic feeding?

Paradoxically, answers to this question are best answered by examination of the postcranial skeleton. Primitive diapsids retained the basic reptilian terrestrial locomotor pattern of side-by-side undulation of the body and tail, increasing the thrust of the limbs, while the powerful tail muscles helped to pull the hindlimb backwards. This basic undulation is also used for swimming, with the limbs trailing. Triassic ichthyosaurs had longer and more flexible necks and bodies than the more dolphin-shaped forms that evolved later. The Triassic forms must have swum by the same undulation of the body and tail that propelled their terrestrial ancestors. A relatively broad, long tail fin, which was an enlargement of the natural keels of the tail, helped generate thrust over most of the animals' length. In contrast, later forms relied mainly on tail movement with force produced by rapid oscillation of an enlarged, bilobate or even crescent-shaped tail fin. This was ideal for maximizing thrust and minimizing drag in steady swimming — perfect for cruising or fast pursuit of prey in open water.

But Triassic ichthyosaurs were not inferior intermediates between terrestrial reptiles and Jurassic ichthyosaurs. The long body and tail permitted large amplitude oscillations and rapid acceleration — useful for ambush predation — and the broad tail gave a burst of power for rapid starts³. Massare and Callaway thus envisage ichthyosaurs as evolving from shallow-water, elongate, crocodile-like predators — in other words, little more than a standard reptile — through a more aquatic but still elongate coastal ambush predator, to the dolphin-like open-water, deep-bodied predators of the open sea. The high degree of adaptation of the intermediates is a particularly pleasing refinement to what was thought to be a well-understood evolutionary development.

The new reports show what insights can be garnered from a marriage of palaeontology and the comparative anatomy of function in living animals. They are, of course, just two of many examples, which together constitute a scientific success story of which Kipling would surely have approved. □

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1. Kipling, R. *Just So Stories for Little Children* (Macmillan, London, 1902).
2. Bodmer, R.E. *J. Zool., Lond.* **219**, 457–467 (1990).
3. Massare, J.A. & Callaway, J.M. *Geol. Soc. Am. Bull.* **102**, 409–416 (1990).
4. Janis, C.M. *Palaeontology* **32**, 463–481 (1989).
5. Sues, H.-D. *J. vert. Paleont.* **7**, 138–144 (1987).
6. Sues, H.-D. *Zool. J. Linn. Soc.* **90**, 109–131 (1987).
7. Rieppel, O. *Phil. Trans. R. Soc. B* **323**, 1–73 (1989).

EVOLUTION

Mountaineering with microbes

Daniel E. Dykhuizen

MICROORGANISMS are well suited to experimental studies of evolution. They have such short generation times that observations of evolutionary change over thousands of generations are possible not only within the lifetime of a scientist, but even within the duration of a grant. Such experiments often give rise to surprising and unexpected results, as demonstrated by the report of Bennett, Dao and Lenski on page 79 of this issue¹.

The authors adapted a population of *Escherichia coli* to growth at 37 °C in a minimal medium by serially transferring the population for 2,000 generations. During this time, the population became well adapted to its environment. From this population a single cell was isolated. The clone derived from the cell was split into six experimental and six control populations, the control populations then being grown in the same environment as before — minimal medium at 37 °C. The experimental populations were grown in the same way and in the same medium, but at

42 °C. After 200 generations, the experimental populations had evolved. In the control populations, there was no measurable change, even after 400 generations. So far there is nothing unexpected in this: rapid evolution in a new environment; none in an environment to which the cells are already well adapted. What might not have been expected is that such similar environments — only 5 °C difference in temperature — lead to different evolutionary responses. The surprise is that the populations evolved at 42 °C were also fitter at 37 °C than the control strains.

Evolutionary biologists have a mental picture of the adaptive process. Fitness is represented by smooth-surfaced mountains, and an adaptive mutation allows a species to climb a little higher on the mountain. If the environment does not change, the species will approach the peak, and as it does so we expect the advantageous changes to become rarer and smaller in effect. In the experiment carried out by Bennett *et al.* this occurred

during the first 2,000 generations at 37 °C such that there is no evidence for any further adaptation after 1,500 generations. So it seems safe to assume that the population is sitting near the top of the adaptive peak.

If the environment is changed, the topography will be remoulded. New peaks will arise from the slopes of old mountains and in place of old peaks there will be valleys. In this case, we could imagine a new, higher peak arising out of the side of the old peak. The population now evolves towards this peak. When this population is returned to the former environment, it would be expected to be less fit, rather than more fit.

The authors offer two hypotheses to explain their results. First, that the mutations selected at 42 °C are not present at 37 °C in sufficient frequency to be selected. This is possible. But then the adaptive path is dependent upon the available mutations even in large populations where most single mutations would be expected within a few tens of generations. If this is generally the case, the metaphor of the adaptive landscape must be restricted to genetic variation currently in the population. Second, that because of the smaller selection coefficient, the mutations did not rise to a substantial frequency at 37 °C. Again this is possible, but the average fitness increase at 37 °C was 4 per cent, half that at 42 °C. Thus, if these mutations dominated after 200 generations in the 42 °C populations, they should have appeared in the 37 °C populations after 400 generations. Where were they?

I offer two more hypotheses. One is that various epistatic mechanisms are involved. For example, assume that two mutations are required for the increase in fitness at 37 °C. Neither of these mutations alone is advantageous at this temperature, but at 42 °C at least one is advantageous by itself and in combination more advantageous. This proposal also has flaws. It would take between 100 and 200 generations for the first mutation to reach frequencies where the second mutation would happen in the same cell. Then there should be a fitness increase in the period from generation 200 to 400, which was not seen. My other hypothesis is that the selection is frequency dependent. When the mutations are rare, they have very little advantage at 37 °C, but considerable advantage at 42 °C. Further explanations can be added to explain the authors' result, but when one starts piling up hypotheses in this way it is hard to stop. Here we have a warning signal that reality does not agree with our picture and investigation of mechanism becomes a necessity.

This is not the only case of unexpected results in experimental studies of evolution. Paquin and Adams² found that a population could evolve such that it became less fit than the starting strain.

Saccharomyces cerevisiae were cultured in a chemostat, a device in which micro-organisms can be continuously grown in a nearly constant environment³. Every time an adaptive mutation swept through the population, strains carrying this adaptation were isolated and competed against ancestral strains, either the one used to start the population or ones carrying previous mutations. Each strain was fitter than the previous one — the strain carrying the first adaptive mutation was fitter than the starting strain, and the strain carrying the second adaptive mutation (and probably also the first) was fitter than the one carrying only the first. But the strain carrying the third adaptive mutation was selected against when set in competition against the starting strain. So selection is not transitive as the mountain model of fitness assumes it to be. To continue the mountain analogy, this result shows that, as the population climbs a mountain, it can change the topography radically even though the physical environment remains constant.

Even in the simplest system — *E. coli* growing in glucose-limited chemostats — unexpected results occur. Chemostats are designed so that a single nutrient limits

growth and the organisms compete for this nutrient. Helling *et al.*⁴ used the system to study the adaptive evolution of *E. coli*, expecting to isolate a series of mutations which made the cells increasingly fitter in this constant environment. The approach should have allowed definition of the path to the peak; instead, the original strain 'speciated' into two separate lineages, both being maintained on what seemed to be a single limiting resource. Closer investigation showed that they had evolved such that the resource was divided — one lineage used the glucose, and excreted compounds on which the other lineage could grow. So the image of a rock-hard mountain decomposes into many fitness landscapes, which in turn continually split into new ones as organisms evolve. □

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1. Bennett, A.F., Dao, K.M. & Lenski, R.E. *Nature* **346**, 79–81 (1990).
2. Paquin, C.E., & Adams, J. *Nature* **306**, 368–371 (1983).
3. Dykhuizen, D.E. & Hartl, D.L. *Microbiol. Rev.* **47**, 150–168 (1983).
4. Helling, R.B., Vargas, C.N. & Adams, J. *Genetics* **116**, 349–358 (1987).

PHYSICAL CHEMISTRY

Shaken, stirred — but not mixed

Irving R. Epstein

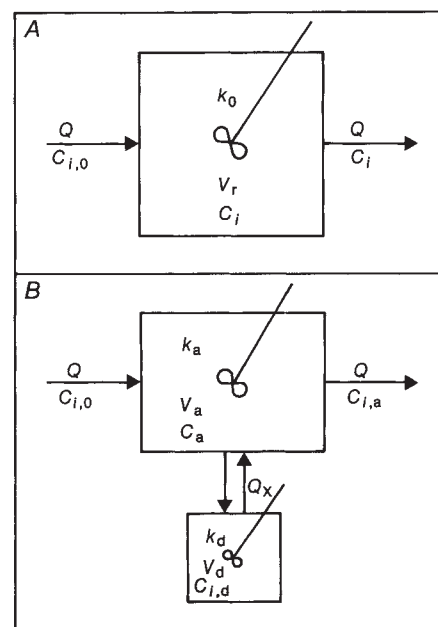
THE glass stirring rod has largely been replaced by magnetic stirrers, shakers, ultrasonicators and other more sophisticated devices designed to produce homogeneous reaction mixtures in chemical systems. Most chemists carry out their reactions in the belief that any residual nonuniformities remaining in their reaction vessels have negligible effects on the results of their experiments. Evidence has been accumulating, however, that in at least some reactions with the kind of highly nonlinear kinetics that leads to several steady states or sustained oscillation, significant concentration gradients arise despite the experimenters' best efforts to eliminate them. Menzinger and Dutt¹ now report quantitative measurements of macroscopic concentration gradients in the oscillatory chlorite–iodide reaction carried out in a stirred continuous-flow reactor. Concentration differences of up to 800 per cent are found between points near to and far from the stirrer shaft.

Chemical engineers have appreciated for some time that such 'irrelevant' details as the placement of input jets, the shape of the stirrer or the orientation of baffles can influence markedly the output of certain reactor processes². Roux *et al.*³ showed the dramatic effect of imperfect mixing in nonlinear chemical dynamics when they

found that, for the bistable chlorite–iodide reaction, the hysteresis limits (as a function of the rate at which reactants are pumped into the reactor) vary significantly as the stirring rate is varied between 550 and 700 r.p.m., a range in which most chemists would have been confident that the reactor was well mixed.

Several theories have been proposed to describe the interaction of mixing effects and chemical kinetics. Two ideas must be kept in mind. First, the effect of inhomogeneities will be most pronounced when the reaction is nonlinear. If the rate of the reaction is proportional to the concentration of reactant X, [X], and if this concentration is 1M in half of the reactor and 3M in the other half, the net (average) rate will be the same as if the entire system was at a concentration of 2M. If, on the other hand, the rate is proportional to [X]², the average rate will be proportional not to 2² = 4, but to ½(1² + 3²) = 5: imperfect mixing increases the net rate by 25 per cent. Second, two kinds of mixing must be accounted for⁴: macromixing, in which parcels of fluid from different inlets must be combined to yield equal bulk compositions; and micromixing, in which molecular mixing occurs for inhomogeneities with sizes of the order of the smallest hydrodynamic turbulent length scale.

In one family of models (see figure),



Models of a stirred-tank reactor. A, Ideal, assuming perfect mixing. B, Macromixing effects included by postulating an active zone (a) which communicates with the input and output reservoirs, and a dead zone (d) which communicates only with a. The C_i s are concentrations of reagent i , k is the rate constant, V is the volume and Q is the flow rate.

which can account for much of the qualitative behaviour, a reactor is pictured as a combination of a well mixed or active (a) zone and a poorly mixed or dead (d) zone. In this model⁵, macromixing (but not micromixing) is described by parameters that determine the rate of exchange between the two zones (Q_x) and their relative volumes (V_a/V_d). Better mixing corresponds to a smaller dead zone. Such a model has been employed in recent efforts to explain the origin of chemical chaos in the Belousov–Zhabotinsky (BZ) reaction. In a somewhat controversial interpretation, Györgyi and Field⁶ suggested that the observed chaotic behaviour⁷, although genuine, arises not from the homogeneous chemical kinetics but from the interaction of these kinetics with imperfect mixing. This view, which was inspired by the inability of chemically reasonable models of the reaction to yield

1. Menzinger, M. & Dutt, A.K. *J. phys. Chem.* **94**, 4510–4514 (1990).
2. Villiermaux, J. *Am. Chem. Soc. Symp. Ser.* **226**, 135–186 (1983).
3. Roux, J.C., De Kepper, P. & Boissonade, J. *Phys. Lett. A* **97**, 168–170 (1983).
4. Boissonade, J. & De Kepper, P. *J. chem. Phys.* **87**, 210–218 (1987).
5. Kumpinsky, E. & Epstein, I.R. *J. chem. Phys.* **82**, 53–57 (1985).
6. Györgyi, L. & Field, R.C. *J. phys. Chem.* **93**, 2863–2867 (1989).
7. Coffman, K.G., McCormick, W.D., Noszticzius, Z., Simoyi, R.H. & Swinney, H.L. *J. chem. Phys.* **86**, 119–129 (1987).
8. Lindberg, D., Turner, J.S. & Barkley, D.J. *chem. Phys.* **92**, 3238–3239 (1990).
9. Nagy-Ungvári, Z., Baumgärtel, H. & Hess, B. *Chem. Phys. Lett.* **168**, 539–542 (1990).
10. Nagypál, I. & Epstein, I.R. *J. phys. Chem.* **90**, 6285–6292 (1986).

truly chaotic behaviour, has been tempered by a recent calculation⁸ which indicates that a plausible model of the BZ reaction can indeed generate chaos under homogeneous conditions. It now seems likely that chaos in chemical systems may have two sources — complex homogeneous kinetics and/or interactions between kinetics and mixing.

The work of Menzinger and Dutt¹ is unlikely to be the last word on the nature of mixing effects in nonlinear chemical kinetics. The 0.25-mm microelectrode that they use to monitor spatial and temporal variations in electrochemical potential gives only very crude spatial resolution; a recent study of pattern formation in the BZ reaction⁹ uses electrodes with micrometre dimensions. By monitoring simultaneously two micro-

electrodes at different locations in the reactor, Menzinger and Dutt find that when the reactor switches from one steady state to another, both the microelectrodes undergo a change in potential at the same time, even though their absolute potentials are quite different. On the other hand, certain chemical systems in a closed reactor show evidence of a transition between stable states that nucleates locally and then propagates through the system¹⁰. But although there are clearly questions still to be resolved, chemists are growing increasingly aware of the danger of assuming that they ever conduct their reactions in media of uniform concentration. □

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IMMUNOLOGY

Tamarin compatibility

Shirley A. Ellis and Andrew J. McMichael

OVER the past few years it has emerged that the major histocompatibility complex (MHC) class I region of human chromosome 6 contains expressed genes in addition to the (so-called classical) A, B and C loci¹. The classical class I genes encode highly polymorphic transmembrane glycoproteins, whose main role is to present peptides from processed foreign antigen to cytotoxic T lymphocytes. To date, three other distinct loci (E, F and G) have been identified² and restriction fragment length polymorphisms suggest that there will be others. The origin and function of these genes have, however, proved elusive. On page 60 of this issue³, Watkins *et al.* provide insights into the possible function of the products of one of these loci, HLA G, from a surprising source — the cotton-top tamarin.

The authors cloned and sequenced all of the MHC class I genes from a sample of 79 unrelated members of this species, and demonstrate that only 11 molecules are expressed. When these were compared with each other, they showed far less variability in amino-acid sequence than is seen between alleles of the class I loci in either man or mouse; ten out of the 11 molecules are more closely related to human HLA G than to classical A, B and C molecules. We must assume that these 'G-like' molecules are functional in the cotton-top tamarin, because the animals can mount cytotoxic T-cell responses. But when they encounter pathogens not found in their natural environment (Epstein-Barr, Rous sarcoma, measles and parainfluenza viruses, for example), they prove to be particularly vulnerable, a phenomenon which may be related to their lack of class I polymorphism.

We cannot assume however that

because the cotton-top tamarin MHC class I appears to function normally, albeit in a limited way, the human HLA G has a similar role. There are several differences to be considered. The two full-length human G (derived) amino-acid sequences that have been reported^{4,5} are identical; together with isoelectric focusing studies^{6,7} this shows that there is probably no polymorphism (at the amino-acid level) at the G locus. The only cells that have been demonstrated to express the HLA G product are extravillous cytotrophoblast from the placenta and some trophoblast-derived choriocarcinoma cell lines. Thus the HLA G molecule is found only on cells that do not express HLA class I A, B or C. Possibly the most striking feature of HLA G which really sets it apart from classical HLA class I is its truncated cytoplasmic tail — the shortened tail is due to a termination codon at the beginning of exon 6 (the first cytoplasmic domain) that results in a smaller expressed product of relative molecular mass (M_r) 39,000. Although it does appear to be anchored in the cell membrane, this missing portion may have implications for the function of the molecule. The 'HLA G-like' molecules seen on the tamarin cells have a normal cytoplasmic tail, and are the size of classical class I molecules, M_r 43,000.

The human placental trophoblast cells, which are located at the maternal-fetal interface, are thought to play a part in the survival of the semiallogeneic fetus. It was believed that these cells formed an MHC-negative 'barrier' against maternal immune surveillance⁸, but recent work has shown that they express high levels of HLA G (refs 5, 7). The function of this molecule is not known — it could be non-immunological, related to the invasive

properties of this tissue, or to the effects of peptide hormones. It may be necessary to have a 'neutral' HLA class I molecule, having a passive but important role, for example, to resist attack by natural killer cells⁹. Selection pressure against polymorphism of an HLA molecule expressed at this extremely sensitive site seems likely, and implies a function fundamentally different from that of the other HLA class I antigens, where a high level of polymorphism plays a crucial part in their immunological activities.

These results raise questions regarding the original nature and role of the ancestral HLA G genes. If the HLA G-like gene products functioned as classical class I molecules in the primate ancestor, they would have needed to 'lose' their polymorphism in adapting to a role in the trophoblast. Alternatively, the ancestral G genes might never have functioned as classical class I molecules; in the tamarin ancestor, accidental deletion of the classical loci may have resulted in an increase in polymorphism at the G locus and a wider tissue distribution. As Watkins *et al.* point out, that these primates are born as stable, bone-marrow-chimaeric twins may be relevant. This might help to compensate for the negative effect of the polymorphism of class I molecules expressed on the trophoblast; females might be tolerant of other parental haplotypes and would therefore be less likely to reject a fetus that carried a foreign MHC class I type.

We need to know therefore if the trophoblast cells in the tamarin express the same class I products as other tissues, or if they show limited or quite different expression. It will also be worthwhile to look more closely at other species that show limited MHC class I polymorphism, perhaps for different reasons (for example the cheetah¹⁰ and the Syrian hamster¹¹), to see if there too evolutionary pressures have acted in a similar way. □

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- Orr, H.T., Koller, B.H., Geraghty, D. & DeMars, R. in *Evolution and Vertebrate Immunity* (eds Kelsø, G. and Schulze, D.H.) (University of Texas Press, 1987).
- Shimizu, Y., Geraghty, D.E., Koller, B.H., Orr, H.T. & DeMars, R. *Proc. natn. Acad. Sci. U.S.A.* 227-231 (1988).
- Watkins, D.I. *et al. Nature* 346, 60-63 (1990).
- Geraghty, D.E., Koller, B.H. & Orr, H.T. *Proc. natn. Acad. Sci. U.S.A.* 84, 945-949 (1987).
- Ellis, S.A., Palmer, M.S. & McMichael, A.J. *J. Immun.* 144, 731-735 (1990).
- Ellis, S.A., Sargent, I.L., Redman, C.W.G. & McMichael, A.J. *Immunology* 59, 595-601 (1986).
- Kovats, S. *et al. Science* 248, 220-223 (1990).
- Head, J.R. & Billingham, R.E. in *Immunological Aspects of Reproduction* (ed Crighton, D.B.) 133-152 (Butterworths, London, 1984).
- Storkus, W.J., Alexander, J., Payne, J.A., Dawson, J.R. & Cresswell, P. *Proc. natn. Acad. Sci. U.S.A.* 2361-2364 (1989).
- O'Brien, S.J. *et al. Science* 277, 1428-1431 (1985).
- Darden, A.G. & Streilen, J.W. *Immunogenetics* 20, 603-607 (1984).

New routes to natural products

John Mann

At one time, the synthesis of complex molecules was the noblest pursuit for an organic chemist. During the past ten years, however, many organic chemists have been lured by the fat research grants and contemporary glamour of bio-organic chemistry. So it is good to see two excellent examples of natural-product synthesis reported by Ley *et al.*¹⁻⁷. The biological interest of one of the compounds (*myo*-inositol 1,4,5-triphosphate (IP₃)) is well known, but that of the other (the anti-parasitic agent avermectin B1a), is also considerable.

There has been enormous interest in the inositol phosphates, in particular the role of IP₃ as a second messenger, ever since their involvement in intracellular signalling was first proposed by Mitchell⁸ in 1975. A number of syntheses of both the natural compounds and certain analogues have been described, but almost all of these commence with *myo*-inositol and proceed via a tortuous sequence of hydroxyl-group protections and deprotections in order to establish the desired phosphorylation pattern. Ley's approach¹⁻³ is conceptually different in that *cis*-3,5-cyclohexadiene-1,2-diol is used as the starting material (top in figure). This is produced from microbial oxidation of benzene by *Pseudomonas putida* and is thus cheap and readily available.

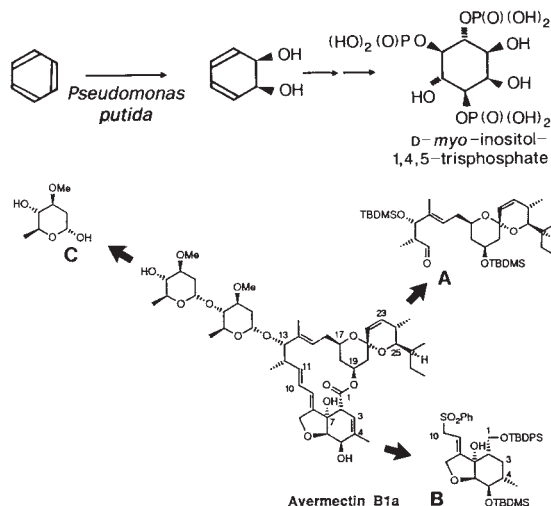
The remaining hydroxyl groups are introduced in a regio- and stereocontrolled manner to produce IP₃ and a large number of analogues. This new route is concise, does not use expensive or esoteric reagents, and provides access to the racemic forms of the compounds, or the pure enantiomers (both natural and unnatural) with only minor modifications. The approach makes a novel range of IP₃-related compounds readily accessible for biological investigation.

The avermectins are mould metabolites first isolated in 1976 from a strain of *Streptomyces avermitilis*⁹. They were shown to have high potency and broad

spectrum of activity against animal parasites. The compounds seem to act at GABA receptors and to maintain the chloride channels in the open form, thus potentiating the inhibitory activity of GABA at neuronal junctions. Several avermectins are now produced routinely using fermentation technology, and ivermectin (the dihydro-analogue of avermectin B1a) is at present sold in more than 60 countries worldwide for the control of both internal and external parasites of livestock.

Notwithstanding this availability, the compounds are challenging targets for synthesis. Avermectin B1a, for example, has 20 stereocentres and a host of delicate functional groups; and to probe the biological activity of these interesting molecules, selected analogues must be prepared. Several research groups have accepted the challenge, but only one complete synthesis¹⁰ of an avermectin has been reported previously.

Ley's new route⁴⁻⁷ (bottom in figure) has the advantages of both conciseness and convergence of the various synthetic routes. The innovative chemistry devised to solve particular synthetic problems is the most interesting aspect of the work.



Synthetic schemes to two natural products (see text).

be of great value for further biological investigation of the way that these compounds work. □

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OCEAN DRILLING PROGRAM

Evolution of the Japan Sea

Leg 127 and Leg 128 shipboard scientific parties

LEGS 127 and 128 of the Ocean Drilling Program spent the summer and early autumn of 1989 drilling at six sites in the Japan Sea (see figure). The goals of these two expeditions aboard the RV *JOIDES Resolution* were to assess the timing, style and dynamics of the opening of this sea and to decipher its subsequent palaeo-oceanographic evolution.

The western Pacific region is characterized by a series of marginal basins bordered by many island arcs. The formation of these marginal seas is intimately linked to the subduction of oceanic crust at adjacent deep-sea trenches. This process fuels the volcanic arcs and creates new oceanic crust and marginal seas through sea-floor spreading and faulting in the back-arc areas between the subduction zone and the continent. The Japan Sea is a large back-arc basin with water depths greater than 3,500 m, separated from the adjacent Pacific Ocean by shallow bathymetric sills of less than 150 m depth. The

geology and geophysical structure of the Japanese Islands point to the presence of continental crust beneath the Japan arc, which in turn calls for a history involving continental rifting and separation of the arc from mainland Asia through back-arc formation of the Japan Sea — a tectonic scenario thought to be responsible for the evolution of many ancient continental margins.

Sixteen years ago, Leg 31 of the Deep Sea Drilling Project (DSDP) attempted to study the history of the Japan Sea but failed to penetrate the sedimentary cover and sample basement rocks, thus leaving many fundamental questions unanswered. So the highest priority of Legs 127 and 128 was recovery of basement rocks, which should record the earliest phases in the evolution of the sea. Leg 127 drilled four locations in the Yamato and Japan basins (sites 794, 795, 796 and 797), where rocks that represent acoustic basement on seismic reflection profiles were

- Ley, S.V., Sternfeld, F. *Tetrahedron Lett.* **29**, 5305–5308 (1988).
- Ley, S.V., Parra, M., Redgrave, A.J., Sternfeld, F. & Vidal, A. *Tetrahedron Lett.* **30**, 3557–3560 (1989).
- Ley, S.V., Parra, M., Redgrave, A.J. & Sternfeld, F. *Tetrahedron* **46**, 4995–5026 (1990).
- Armstrong, A. & Ley, S.V. *Synlett* 323–325 (1990).
- Diez-Martin, D., Grice, P., Kolb, H.C., Ley, S.V. & Madin, A. *Synlett* 326–327 (1990).
- Armstrong, A., Ley, S.V., Madin, A. & Mukherjee, S. *Synlett* 328–330 (1990).
- Frod, M.J., Knight, J.G., Ley, S.V. & Vile, S. *Synlett* 331–336 (1990).
- Mitchell, R.H. *Biochim. biophys. Acta* **415**, 81–147 (1975).
- Davies, H. G. & Green, R. H. *Natural Products Rep.* **3**, 87–121 (1986).
- Danilshesky, S.J., Armistead, D.M., Wincott, F.E., Seilnick, H.G. & Hungate, R. *J. Am. Chem. Soc.* **111**, 2967–2980 (1989).

penetrated for the first time and complete overlying sedimentary sections were recovered. Leg 128 followed by drilling at two topographic highs on the sea floor, including site 798 on Oki Ridge which yielded a key Pliocene and Pleistocene palaeoceanographic reference section for the sea and an unusually clear record of explosive volcanism during this period. Site 799 was located in the Kita-Yamato Trough, a sediment-filled graben (rifted valley) which splits the Yamato Rise. This trough is thought to represent a failed rift within a fragment of continental crust that was isolated during initial back-arc spreading. It is thought to be an ideal setting for the formation of volcanogenic massive sulphide deposits, and it represents the first CDP site selected specifically to test ideas about deep-sea ore genesis. Drilling at this site produced a sequence representing the Lower Miocene through to the Holocene, which will enhance our understanding of metaliferous back-arc environments. Leg 128 also returned to site 794 in the northern Yamato Basin to perform two downhole geophysical experiments designed to probe the structure and character of the crust and upper mantle beneath the Japan Sea.

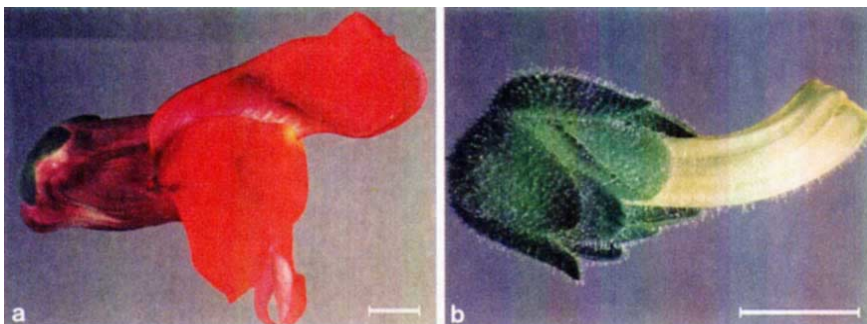
Drilling by Leg 127 at sites 794, 795 and 797 established that the volcanic basement rock encountered at these sites is of Lower Miocene age. Lithological sections representing acoustic basement at site 797 included interlayered coarse turbidite sands and basalt sills and flows, whereas the basement at site 795 was formed from brecciated basaltic lavas. Thus these facies probably explain the thick oceanic crust indicated by seismic velocity profiles beneath the Yamato Basin. The combined drilling of Legs 127 and 128 at site 794 penetrated 190.5 m into a basal series of stacked tholeiitic dolerite and basalt sills and flows representing the early Miocene floor of the northern Yamato Basin. Deep-water microfossils recovered from small sediment sequences intercalated within volcanic rocks at site 794 attest to the rapid subsidence of the Yamato Basin shortly after initial back-arc rifting, a process confirmed by the results from sites 797 and 799. Indeed, all evidence obtained by Legs 127 and 128 indicates that the Yamato Basin was formed by extremely rapid subsidence during the early Miocene (probably before 19 Myr ago) accompanied by intense volcanic activity.

The sedimentary sequences overlying basement rocks at all six sites in the Japan Sea show striking regional similarities and form five distinct lithological associations which record major tectonic and palaeoceanographic changes during evolution of the sea. These include (1) Lower Miocene delta-front sands and siltstones rich in plant debris, which were

Changing the flowers

WITH the report by Sommer *et al.* of the cloning of the snapdragon *deficiens* gene (*EMBO J.* 9, 605–613; 1990), and that by Yanofsky *et al.* of the cloning of the *agamous* gene in *Arabidopsis thaliana* (page 35 of this issue), the study of plant development has taken a big step forward.

The picture, reproduced from the *EMBO Journal*, shows (a) a normal flower of the snapdragon (*Antirrhinum majus*), and (b) one produced by a plant with a mutation at the *deficiens* locus. The petals of the mutant flowers are replaced by a second ring of sepals, the leaf-like organs that form the outer whorl of flowers of this kind, and the stamens are transformed into carpels, which form the tubular structure protruding from the two rings of sepals.



Like *deficiens*, *agamous* is classed as a homeotic gene, because its mutations cause the transformation of one type of flower organ into another. But the effect of *agamous* mutations is quite different from that of *deficiens* mutations: the six stamens of the mutant flowers are replaced by six extra petals, and the carpels are replaced by a whole new flower. The new flower suffers from the same homeotic transformation, so that a repeating pattern of sepals and petals is generated.

The normal flowers of both snapdragon and *A. thaliana* consist of four concentric whorls of organs: an outer whorl of sepals, followed by whorls of petals, stamens and finally carpels. The mutations that cause transformations of these organs differ in important respects from the better-known homeotic mutations of the fruitfly *Drosophila melanogaster*. The fruitfly homeotic mutations cause discrete transformations of one segment of the fly's body into another, so that a segment is exactly replicated and the segment number is not changed; in the *agamous* mutation, by contrast, the number of petals in the transformed third whorl is six, the same as the number of stamens that would normally be present in this whorl, and not the four that would be expected by a precise reiteration of the second whorl.

The sequences of the *deficiens* and *agamous* protein products show a region of roughly 56 amino acids where they are very similar, both to one another and to two known transcription factors, human serum response factor and the yeast protein MCM1. In the case of serum response factor at least, the conserved region is known to fall within the DNA-binding domain of the protein. So the strong presumption is that flower homeotic gene products form a class of DNA-binding regulatory proteins linked by their similar DNA-binding domains, much as the fruitfly homeotic genes are linked by their 'homeobox' domains.

Geoffrey North

deposited rapidly during initial back-arc rifting and basin subsidence; (2) widespread Middle Miocene siliceous and carbonate-rich hemipelagic claystones and volcanic tuffs, marking a period when the rate of basin subsidence outpaced the rate of sediment accumulation in the sea; (3) Upper Miocene claystones and porcellanites; (4) Pliocene diatom oozes representing evidence of climatic cooling, upwelling and increasing primary productivity in the sea; and (5) light/dark rhythmic Plio-Pleistocene sediments, reflecting rapid oscillations in the oceanographic behaviour of the Japan Sea from stagnant, near-anoxic conditions to the fully oxic and hyperventilated conditions exemplified by the sea at present. Significantly, the latter cyclic sequences are best

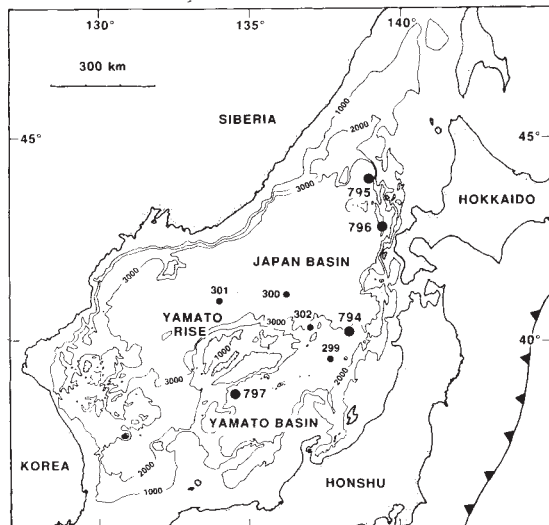
developed in the upper Pleistocene sediments which represent the past 400,000 years, during which time basin sills and climatically induced periods of low sea level may have periodically isolated the Japan Sea from the open Pacific.

The relatively recent tectonic emplacement of shallow bathymetric sills or straits separating the Japan Sea from the Pacific Ocean is one manifestation of a convergent plate boundary apparently developing along the eastern margin of the sea, as evidenced by uplifted en echelon ridges, thrust faults and large compressional earthquakes. Leg 127 drilling at site 796 revealed that gravity-driven deposition filling the northwestern Japan Basin stopped, and uplift of the Okushiri Ridge above the basin floor subsequently

began, in the latest Pliocene (1.8 Myr ago), at the same time that widespread deformation occurred along the entire eastern margin of the Japan Sea. This activity might represent an initial stage in the now continuing compressive destruction of the sea.

Although deep-sea drilling has been very successful in unravelling ocean history by allowing direct study of rocks and sediments in cores, the technique is limited to the upper 1.5 kilometres of

floor in volcanic basement rock. The emphasis in this experiment was on long-period signals, which probe larger scales and deeper structure. Following an experiment that essentially monitored instrument performance and analysed local crustal structure, the seismometer is now accumulating long-term data on natural earthquakes, which will ultimately be used to create a three-dimensional image of the crust-mantle configuration in this region.



Map of the Japan Sea, showing locations of ODP Leg 127 sites (dots), Leg 128 sites (circled dots) and DSDP sites (triangles). ODP site 794 was occupied during both Legs 127 and 128. Bathymetry in metres.

ocean crust. Leg 128 carried out two multi-ship geophysical experiments at site 794 that bridged the gap between direct observations of the upper crust from drilled cores and remotely sensed data on the structure of the deeper crust and upper mantle. The Japan Sea was ideally suited to these experiments because the structure of the deep crust and mantle varies laterally from the Pacific plate (in front of the subduction zone) through the Japanese Island arc to the Japan Sea behind it. These variations reflect the dynamics of the subduction process.

The first experiment involved installation of a specially designed digital broadband seismometer at 715 m below the sea

The second experiment comprised active-source electrical measurements designed to give high-vertical-resolution profiles of the electrical resistivity in the crust and upper mantle. Resistivity measurements can yield information on crustal temperatures and the presence of fluids or partial melts. Resistivity observations at site 794 were made by placing an array of electrodes in a drilled hole and having a supporting ship apply an electrical charge to the water column at increasing distances from the drill hole. The results will yield new estimates of electrical resistivity to a depth of 10 km beneath the Japan Sea.

The results as a whole indicate that the marginal basin of the Japan Sea seems to be in a mature stage of evolution despite its recent birth in the late Oligocene to early Miocene. Indeed, the relatively young age of the sea has allowed all of the key elements in the back-arc spreading process to be deduced from samples of sea-floor basalts and sediments, along with the history of the water mass.

Although the data collected during Legs 127 and 128 have yet to be fully analysed, it is clear that the Japan Sea now stands as one of the best documented back-arc basins in the world and provides a potential model for interpreting the rifting and mountain-building processes that characterize many modern and ancient continental-arc settings. □

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Leg 128. Co-Chief Scientists: James C. Ingle, Jr. (Stanford University) and Kiyoshi Suyehiro (University of Tokyo). ODP Staff Scientist: Marta T. von Breyman (ODP, Texas A&M University). Also, James Bristow (Lamont-Doherty Geological Observatory), Lloyd H. Burckle (Lamont-Doherty Geological Observatory), Jacques Charvet (University of Orleans), Barry A. Cragg (Scottish Marine Biology Association), Peter DeMenocal (Lamont-Doherty Geological Observatory), Robert B. Dunbar (Rice University), Karl B. Föllmi (ETH-Center, Zurich), John R. Griffin (University of Nebraska), Kurt A. Grimm (University of California, Santa Cruz), Yozo Hamano (University of Tokyo), Naoshi Hirata (Chiba University), Peter Holler (University of Kiel), Caroline M. Isaacs (US Geological Survey), Michio Kato (Kanazawa University), Richard Kettler (University of Nebraska), Tara Kheradvar (Stanford University), Klaus A.O. Krumsiek (University of Köln), Hsin-Yi Ling (Northern Illinois University), Ryo Matsumoto (University of Tokyo), Jay P. Muza (Florida State University), Ronald J. Parkes (Scottish Marine Biological Association), André Poulet (University of Orleans), Steven D. Scott (University of Toronto), Rüdiger Stein (Justus-Liebig-Universität) and Anne A. Sturz (Scripps Institute of Oceanography).

Sun and air

ONE of the neatest ways of trapping solar energy is the solar pond. This contains ordinary water, with a layer of denser salt water underneath it. The slow upward diffusion of the salt produces a stable density gradient in the pond; convection cannot occur. Solar radiation penetrates the water and is absorbed by the blackened bottom of the pond. The heat cannot convect upwards; it leaks upwards by conduction only slowly; the bottom of the pond can almost reach boiling point.

In this connection Daedalus recalls the Valley of Death in Java, into which carbon dioxide is steadily released from some subterranean volcanic source. The gas forms a layer on the valley floor. Men and animals who enter the valley (except, possibly, giraffes) are suffocated. But plant life flourishes luxuriantly.

Gases, especially heavy ones, have even lower thermal conductivities than liquids. So, says Daedalus, a gaseous nonconvecting 'solar pond' on the Valley of Death principle should be even more efficient. DREADCO's engineers are now converting an empty reservoir into a prototype. They are roofing the top with a supported web of transparent sheeting, and the bottom with reflective aluminized film. They will then pour carbon dioxide, or some even heavier gas, over the floor of the reservoir. A grid of blackened absorber pipes, supported just off the bottom, will be heated by solar radiation entering from above and reflected from below, while being excellently insulated by the surrounding nonconvective density-stratified gas. It will heat up until convection can just overcome the density gradient against it.

With carbon dioxide, this should happen at 180 °C; sulphur dioxide could give 390 °C and dichlorodifluoromethane could give almost 1,000 °C. Although these limits are unlikely to be reached in practice, the solar gas-pond could clearly generate high-grade heat and power while providing a splendid and ecologically virtuous dumping ground for gaseous industrial nasties. It would, of course, slowly lose efficiency as the stratified gas diffused towards uniform composition. Fortunately, the diffusion-time increases as the square of the mixing distance. A solar gas-pond 40 metres deep would take years to mix completely. Slow removal of the central stratum, while feeding in fresh heavy gas at the bottom and air at the top, could maintain the density gradient indefinitely.

A carbon dioxide solar gas-pond should also act as a wonderful forcing house for tropical vegetation. Its high temperature and insect-suffocating, photosynthesis-encouraging atmosphere should enable coconut palms and breadfruit trees to flourish effortlessly in the bleakest of temperate zones.

David Jones

Brilliant pebbles won't do

SIR—In their recent Commentary¹, G. Canavan and E. Teller conclude that singlet space-based interceptors (SBI) are "... effective in the near-term and mid-term ..." and that they can be made adequately survivable against achievable Soviet reactions by "hardening, evasion, and decoy in combination ...". These conclusions stem from the constraints imposed by the authors on the Soviet response. Unaccountably, they assume the Soviets will be able to reduce missile burn time T only to 300 seconds or so. But the Soviets already deploy SS-25 missiles with $T = 180$ seconds, corresponding to an availability of 1.1% for the SBI. Subtracting the SBI "release time" of 50–100 seconds would further reduce the effectiveness of SBI, according to Lt Gen. George L. Monahan's formal response to the US Senate Appropriations Committee on the SDI programme.

Ten warheads deployed on silo-based single-warhead SS-25s within a region a few hundred kilometres or less across would require $10/1.1\% = 900$ brilliant pebbles to be able to assign even a single interceptor to each missile. As few as 1,000 ICBM warheads would thus need 90,000 brilliant pebbles—a clear demonstration that the defence is not cost-effective at the margin.

The Senate Appropriations Committee went on to ask whether the SDI phase I architecture would be capable of destroying more than a few SS-25s in the boost phase. Monahan answered "No. The phase I SDS (Strategic Defense System) does not provide continuous coverage of the SS-25 deployment region given the available time (SS-25 burn time minus surveillance/command and control timeline)."

If the burn time of a new solid-fuel missile were reduced to $T = 100$ seconds, there would be no intercepts at all. The launch weight of a booster designed to deploy its single warhead in 100 seconds would be about 5% above that of a missile of normal burn time².

Canavan and Teller dismiss an even more important and less costly countermeasure: "Attrition during peacetime ... While anti-satellites would be effective in that role, their use might be viewed as an attack, so the main danger in this case would probably come from ground-based lasers." But the Soviets already launch hundreds of test missiles a year without our viewing them as an "attack". A deployment of SBI could provoke the building of a similar number of Soviet small ASAT weapons, with 40-kilogram homing warheads. Only about 3.5 kilometres per second need be supplied by a two-stage rocket to lift the ASAT to 400-kilometre altitude at precisely the time an

SBI is expected at the intercept point. Launching such a small, slow rocket in Kazakhstan could not be regarded as an attack on the United States; it could not reach us, but it could reach our space weapons overflying the Soviet Union.

The key points in making the ASAT clearly cheaper^{3,4} and simpler than the brilliant pebbles are that the rocket mass required to launch a 40-kilogram ASAT homing head to intercept altitude is a factor of seven smaller than that required to launch into orbit a 40-kilogram SBI (bearing a 5-kilogram homing head), and the much easier task of an ASAT performing its job within full view of ground-based radar and optical sensors, designators and compound intelligence. This ground-based support would allow the ASAT to identify and destroy manoeuvrable, decoyed SBI, or (equally effective) to provoke them to manoeuvre and dispense decoys

so that they will be vulnerable weeks or months later.

If helpful, intercepts could be timed so that the intercept takes place in daylight while an observation point on the ground is in the dark. With a phase-I SDI deployment of 4,000 SBI, an SBI would fly within 50 kilometres of the vertical over the ASAT launch site about 20 times an hour, so there are many opportunities for intercept. A deployment of 40-kilogram brilliant pebbles could be defeated at much lower cost and thus would impair rather than add to US security.

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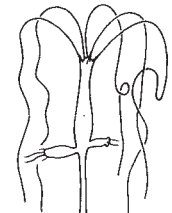
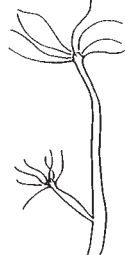
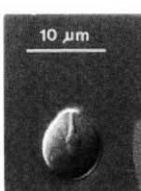
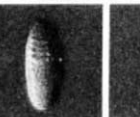
1. Canavan, G. & Teller, E. *Nature* **344**, 699–704 (1990).
2. Bloembergen, N. & Patel, C. K. *Rev. Mod. Phys.* **59**, No. 3 Part II S30–32 (1987).
3. Speed, R. D. Lawrence Livermore National Laboratory Report UCRL-ID-103669 (1990).
4. Garwin, R. L. *Nature* **344**, 301–302 (1990).

Identity crisis

SIR—Hydra are used frequently in research and teaching^{1,2}, but identifying them is extremely difficult because names have been used inconsistently. Recent work has clarified the names and identities of the five species described in Europe^{3–5}. The nematocysts ("stingers") provide the most certain means of identification, and the morphological characters of the body of hydra, as shown in the figure, provide

simple guides for identifying the species. *H. viridissima* is immediately identifiable by its green colour, and *H. oligactis*'s identity is revealed by two precocious tentacles on young buds. Identification of the other three species on the basis of morphological criteria is more difficult because the lengths of the tentacles and body vary with culture conditions.

One reason for confusion among names is that the species of hydra most widely used in laboratory research has been

	<i>H. viridissima</i> PALLAS 1766	<i>H. oligactis</i> PALLAS 1766	<i>H. vulgaris</i> PALLAS 1766	<i>H. circumcincta</i> SCHULZE 1914	<i>H. oxycnida</i> SCHULZE 1914
Correct binomial					
Recent synonyms	<i>H. viridis</i>	<i>H. fusca</i>	<i>H. attenuata</i>		
					
Colour	green				
Number of tentacles on young bud	5–6	2		5–6	
Length of body	moderate (5–10 mm)	large (8–15 mm)	moderate (5–10 mm)	small (3–7 mm)	very large (10–20 mm)
Length of tentacles relative to body	1/2–1	2–4	1/2–1	1/2–1	1/2–1
Stenoteles					
Holotrichous isorhizas					

frequently and incorrectly called *H. attenuata*. This name is no longer considered valid and the species should be known as *Hydra vulgaris* Pallas 1766. Another reason for confusion is that all the European species of hydra have been known by a plethora of names, most of which have been applied to more than one species. The unruly nomenclature has obscured the fact that there are rather few species of hydra and that these are easily distinguishable.

Similar general types of hydra occur on all continents, although the names and the number of species varies. *H. oligactis* and *H. viridissima* are cosmopolitan. In North America, *H. littoralis* and *H. carnea* among others, and in Japan, *H. magnipapillata*, closely resemble *H. vulgaris* of Europe. *H. hymanae* and *H. utahensis* from North America are similar to *H. circumcincta*. Whether some of these are synonyms is not yet known.

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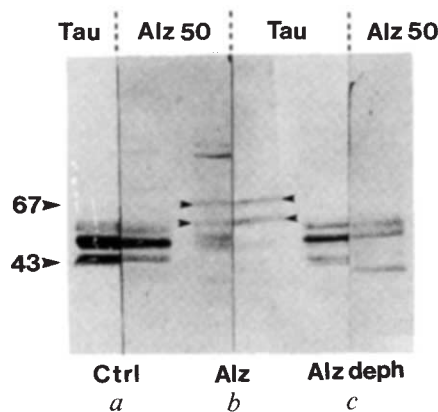
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1. Tardent, P. *Coelenterata. Cnidaria*. (ed. Seidel, F. Morphogenese der Tiere, L. 1: Al) 69–415 (Fischer, Jena, 1978).
2. Lenhoff, H.M. (ed) *Hydra: Research Methods* (Plenum, New York, 1983).
3. Campbell, R.D. *J. Linn. Soc. Lond.* **91**, 253–263 (1987).
4. Campbell, R.D. *J. Linn. Soc. Lond.* **95**, 219–244 (1989).
5. Holstein, T.W. *Hydrozoa* (ed. Schwoerbel, R.J. *Süsswasserfauna von Mitteleuropa* 1.1), (Fischer, Stuttgart, 1990).

Tau marker?

SIR—The Alz-50 monoclonal antibody recognizes a neuronal antigen, termed A68, exclusively in Alzheimer brain tissue¹. This antigen, of relative molecular mass 68,000 (68K), is also detected in the cerebrospinal fluid² and in the olfactory epithelium from patients with Alzheimer's disease³, and it has been suggested that Alz-50 might help *in vivo* diagnosis. It was then shown that Alz-50 also cross-reacts with tau proteins⁴, the main antigenic component of the paired helical filaments that accumulate in degenerating neurons during Alzheimer's disease, suggesting that A68 might be an aberrant form of tau. We have shown that two tau proteins of M_r 64 and 69K, tau 64 and 69, are specifically detected in brain areas affected by the neurofibrillary degeneration⁵. These proteins arise from an abnormal phosphorylation of tau, as demonstrated by the decrease in their mass after alkaline phosphatase treatment⁶. The crossreactivity of Alz-50 with tau proteins causes us to suspect that Tau 69 and A68 are the same protein.



Binding of Alz-50 and anti-tau on immunoblots of frontal cortex from a control (a) and a patient with Alzheimer's disease before (b) and after (c) alkaline phosphatase treatment. The dephosphorylation was performed as described in ref. 5, and details are available on request from the authors.

Alz-50 was reacted against immunoblotted total protein extracts from different control and Alzheimer's brain areas. These immunoblots reveal that Alz-50 is essentially directed against tau 64 and 69 (see figure) and that no protein other than the pathological tau proteins are detected in this molecular-mass range. The experimental dephosphorylation of tau 64 and 69 results in the formation of 50–60K products, which are still labelled by Alz-50. Immunoblots of two-dimensionally resolved proteins corroborate these findings.

Our results strongly suggest that A68 is an abnormally phosphorylated tau protein. Because the expression of tau 69/A68 is correlated with neurofibrillary degeneration and intellectual impairment⁶, Alz-50 marker might be used to help diagnosis of the disease. Moreover, the further study of tau 69/A68 could help track the biochemical dysfunctions that lead to neuronal death and dementia.

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1. Wolozin, B.L., Pruchnicki, A., Dickson, D.W. & Davies, P.A. *Science* **232**, 648–650 (1986).
2. Wolozin, B. & Davies, P. *Ann. Neurol.* **22**, 521–526 (1987).
3. Talamo, B.R. *et al. Nature* **337**, 736–739 (1989).
4. Ksiezak-Reding, H., Binder, L.I. & Yen, S.-H. *J. Biol. Chem.* **263**, 7948–7953 (1988).
5. Flament, S., Delacourte, A., Hémon, B. & Défossez, A. *J. Neurol. Sci.* **92**, 133–141 (1989).
6. Flament, S. *et al. Acta Neuropath.* (in the press).

Correction

In the Commentary article "Duesberg, HIV and AIDS" (*Nature* **345**, 659–660; 1990) it is said that homosexuals in the United States have a 900-fold higher risk of developing Kaposi's sarcoma. As stated in the same paragraph, the 900-fold higher incidence referred to HIV-positive homosexuals compared to HIV-negative homosexuals. □

Mutagen test

SIR—It is commonly assumed that environmental mutagens are responsible for most mutations in germline and somatic cells, and that mutations arising through endogenous processes such as depurination, deamination, replication error and transposition are comparatively infrequent. With the development of direct sequencing methodology, enough mutational events can be examined to address this issue more directly. Mutagens should produce different mutation patterns in populations exposed to different environments, as each mutagen has its own fingerprint of mutation¹. By contrast, endogenous processes could produce an essentially invariant pattern of mutation. I find the spectrum of germline mutations from a particular sample to be similar to the spectrum of somatic mutations associated with the p53 and retinoblastoma genes, suggesting that endogenous processes are dominant in these cancers. I propose that tumour-suppressor genes could be useful epidemiological tools for determining the exceptions where environmental mutagens do predominate.

A meaningful comparison of the pattern of mutation requires genes where the observed pattern faithfully reflects the underlying pattern of mutation. From a population-based study of Caucasian families with haemophilia B residing in the midwestern United States and Canada (see table), we deduced² that the observed mutations do approximate the underlying pattern (corrected for biases of mutant ascertainment) of recent germline mutations in factor IX. A similar pattern of mutation occurs in Asians³.

The tumour-suppressor genes offer an opportunity to examine the pattern of somatic mutation *in vivo*, without the artefacts induced by tissue culture. By contrast, dominant oncogenes, such as *ras*, are not good models for examining the pattern of spontaneous somatic mutation because only a few mutations will produce disease. Mutations in the p53 and retinoblastoma genes have recently been published (see table), and the pattern of somatic mutation in colon, brain, breast, and retina is similar to the pattern of germline mutation. Lung tumours are an exception to the general pattern ($P < 10^{-4}$); most cases occur in smokers, exposed to enormous amounts of inhaled mutagens.

These data suggest that the underlying

1. Suzuki, D.T., Griffiths, A.J.F., Miller, J.H. & Lewontin, R.C. (eds) *An Introduction to Genetic Analysis* 333–335 (Freeman, New York, 1986).
2. Koeberl, D.D. *et al. Am. J. Hum. Genet.* (in the press).
3. Baker, S.J. *et al. Science* **244**, 217–221 (1989).
4. Bottema, C.D.K., Kettering, R.P., Yoon, H.-S. & Sommer, S.S. *Am. J. Hum. Genet.* (in the press).
5. Nigro, J.M. *et al. Nature* **342**, 705–708 (1989).
6. Yandell, D.W. *et al. N. Engl. J. Med.* **321**, 1690–1695 (1989).
7. Takahashi, T. *et al. Science* **246**, 491–494 (1989).
8. Iggo, R., Gatter, K., Bartek, J., Lane, D. & Harris, A.L. *Lancet* **335**, 675–679 (1990).

DISTRIBUTION OF POINT MUTATIONS

Mutational type	Germline cells		p53 Colon	Neoplastic cells		p53/Rb Lung
	Factor IX Caucasians*	Factor IX Asians		p53 Bn/Br	Rb Retina	
Transitions (Transitions at CpG)	31 (12)	10 (6)	11 (6)	6 (3)	5 (3)	0 (0)
Transversions	5	4	2	0	1	8
Micro-deletions/insertions	2	2	2	0	2	1
Ref.	2	3	4,5	5	6	5-8

*The data approximate the underlying pattern of germline mutation in factor IX. Transitions at CpG are elevated 24-fold relative to other transitions (ref. 2). When the subset of Caucasians with severe disease was compared with the Asian sample, the patterns were essentially identical. The excess of deletions in retina cells reflects the large and highly redundant nature of the retinoblastoma (Rb) protein. Five of the six base substitutions produce nonsense or splice junction defects, implying that the more numerous missense mutations are underrepresented because they generally do not cause disease.

pattern of germline or somatic mutation will commonly be the same. It seems most likely that there is a strong selective pressure for endogenous control of the mutational process, rather than a ubiquitous and prominent mutagen. For germline mutation, the rate may be constrained by opposing evolutionary forces. Too little germline mutation would result in extinction of the species, as the level of variation in the population would be insufficient to

adapt to environmental changes. Conversely, more than the requisite amount would increase the morbidity of the species as virtually all disease has a genetic predisposition.

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Sensing reducing gases

Sir—The recent report¹ of a novel thin-film electrochemical cell for energy conversion prompts us to describe some work we have been carrying out using a related concept for the purpose of detecting reducing gases such as hydrogen and carbon monoxide in air.

Our cell is shown schematically in Fig. 1. Its solid components are a porous oxide substrate (polymer substrates, as described in ref. 1 are also useful) and two electrodes of dissimilar metals which are deposited by d.c. sputtering. For an electrolyte we depend entirely on a film of

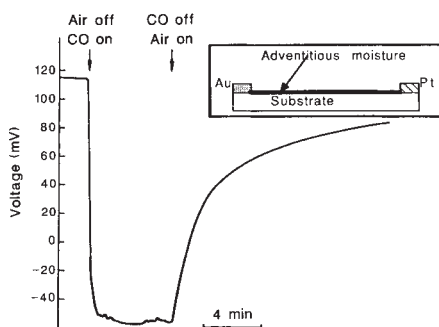


FIG. 1 Voltage response of WO_3 to 1% CO in air. Open-circuit potential from a cell based on a porous pellet of tungsten trioxide with platinum sputtered on to one flat surface and gold on the other. The potential difference was monitored via a Keithley model 614C electrometer with the platinum electrode connected as positive. The atmospheric compositional change between air and air containing 1% carbon monoxide at the points marked gave rise to a change of over 150 mV in the open circuit potential of the cell. Inset: schematic of the thin-film electrolytic cell.

moisture adventitiously absorbed from the atmosphere onto the surface of the substrate between the electrodes. With electrodes of platinum and gold, respectively, and the cell exposed to air in the relative humidity range between around 20 and 100% at room temperature a substantial open circuit potential (up to 500 mV) is generated. Operation requires the presence of both oxygen and water vapour.

The geometry of the three-phase interface involving a film of adsorbed moisture is ideally suited for sensing gas reactions: changes in open circuit potential result when the cells are exposed to small concentrations in air of a range of gases including hydrogen, carbon monoxide, ammonia, hydrogen sulphide and ethanol vapour (Fig. 1). The open circuit potential change of the cell increases approximately as the log of the concentration of the reducing gas in an air ambient (Fig. 2).

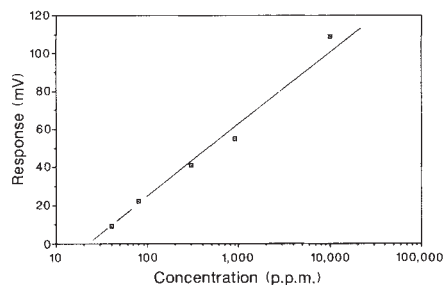
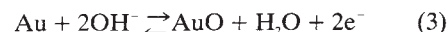
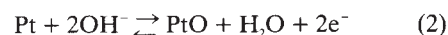
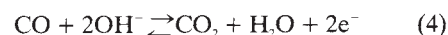


FIG. 2 Variation in the magnitude of the change in potential of a cell based on a porous pellet of tin dioxide, with one electrode platinum and the other gold as a function of the logarithm of the concentration of hydrogen in air.

We believe the mechanisms of response as involving changes in "mixed potentials"², which are different on different metals. An electrode at open circuit adopts a potential (mixed potential) such that the rate of all the anodic and cathodic processes balance. In air, in the absence of any other gases, the processes are the reduction of oxygen, and the reduction and oxidation of the metal:



The balance between reactions (1) and (2) establishes a different potential from that between reactions (1) and (3), hence accounting for the open-circuit potential difference of the cell. The presence of a fuel gas such as CO introduced the possibility of a different reaction:



which we surmise affects the mixed potential predominantly on the platinum electrode rather than on the gold, and hence gives rise to a change in the output of the cell.

As in the case of the thin-film electrolyte fuel cell¹, our device exhibits the convenient attributes of operating at room temperature and functioning without the need to separate the analyte (fuel) gas from the oxidant. The key role of reaction kinetics is also common to both applications.

For sensor applications the cell we have described offers a number of useful features: independence from the need for a reference volume leads to simplicity; room-temperature operation renders the detection of potentially flammable gases such as hydrogen a safer prospect and the open circuit potential in air provides a useful 'condition safe' indicator.

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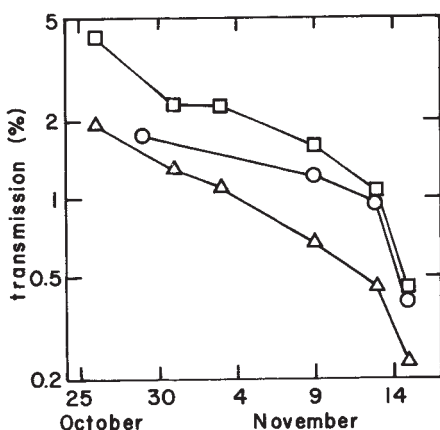
1. Dyer, C.K. *Nature* **343**, 547-548 (1990).
2. Charlot, G., Badox-Lambling, J. & Trémillon, B. *Electrochemical Reactions: The Electrochemical Methods of Analysis* 271-307 (Elsevier, Amsterdam, 1962).

Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. They need not arise out of anything published in *Nature*. In any case, priority will be given to letters of fewer than 500 words and five references. □

Radiation risk

SIR—There is a significant increase of ultraviolet levels in the Antarctic during the presence of the 'ozone hole', and consequently there is concern for Antarctic life forms which may have developed only minimal defences against this radiation^{2,3}. We have previously pointed out⁴ that the problem is potentially most acute under the vast sea ice cover surrounding Antarctica, for the sea ice is particularly transparent when the ozone hole occurs. Because we did not know the ultraviolet properties of the ice, our argument relied



Seasonal development of the transmission of sea ice at wavelengths of 300 nm (ultraviolet-B, triangles) 350 nm (ultraviolet-A, squares) and 650 nm (mid-visible, circles).

on an extrapolation from data in the visible part of the spectrum. Here we report measurements showing that the transmission of sea ice shows an even larger seasonal variation in the ultraviolet than in the visible spectrum.

The experiments were performed on 1.7-m-thick first-year sea ice in McMurdo Sound, using a technique described elsewhere⁵. The measured development of the transmission of the ice at three wavelengths is shown in the figure. All three curves show a substantial decrease during the spring, resulting from the development of a turbid surface layer left behind after brine pockets in the ice drain⁶. Surprisingly, the development is stronger in the ultraviolet than in the visible, for which we do not yet have a satisfactory explanation.

Algae residing near the ice-water interface are the first major biological popula-

tion to experience the radiation entering the ice. These algae have been estimated to account for up to 30% of the primary food production in the ice covered regions of the southern ocean⁷, and they are known to be sensitive to ultraviolet radiation². We have estimated the level of flux falling on these algae as the product of the sea ice transmission of the figure and the surface flux measured at Palmer Station during the 1988 spring¹. In the latter half of October, the under-ice flux rises as high as 10^{-3} W m^{-2} in a 10-nm band centred on the more damaging ultraviolet-B region, up from about $0.3 \times 10^{-3} \text{ W m}^{-2}$ before the appearance of the ozone hole. The 1988 ozone depletion was rather weak, and for the stronger depletions of 1987 and 1989 the October under-ice flux in the same band⁸ would rise to about $5 \times 10^{-3} \text{ W m}^{-2}$. Later in the season, the turbid surface

layer shields the ice, so that in the height of summer the flux in this band is very much lower, about $5 \times 10^{-5} \text{ W m}^{-2}$. Thus the yearly dose at the base of the ice is overwhelmingly dominated by the October flux, with the consequence that the ice algae will have experienced more than a tenfold increase of ultraviolet-B dose in recent years compared to the pre-1975 levels.

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Cortical maps

SIR—Durbin and Mitchison¹ suggest an explanation for cortical maps and their distortions. If their purpose is to explain the biological conditions for the formation of two-dimensional brain maps, a theoretical description of the lateral interactions between neural cells would require the use of spatio-temporal partial differential equations^{2,3}. But to implement powerful artificial 'neural' computing structures (which can form dimensionality-reducing mappings and abstractions), the self-organizing process is better described by computational algorithms that simultaneously update sets of neighbouring neural units⁴.

Contrary to what Durbin and Mitchison claim, the equation they use (see their legend to Fig. 2) is not based on Hebb's law of synaptic plasticity; the first term would need a more lengthy justification⁵. Their second term was introduced "to make neighbours have similar receptive fields", but they did not explain what kind of biological adaptive process would account for this effect. This term in fact seems to stem from the physical analogy of stress-strain relations in an elastic ring or net⁶. In the other works cited by Durbin and Mitchison, on the other hand, lateral interaction has been used to activate neighbouring cells to learn from the same input, which is a different and natural assumption.

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Kohonen's book and Swindale's work. However, we should perhaps have spelt out more clearly the ancestry of our simulation. Of course, the main goal of our paper was to consider wiring minimization as an organizing principle for maps of many variables, and to relate this to self-organizing models. We believe this is a new approach to cortical mapping.

We are surprised at Kohonen's rather critical comments about the self-organizing model we used, since it operates in a similar fashion to his own. Both work at a fairly abstract level, which we believe was appropriate for our paper. As he remarks, the first term in our equation, though not directly hebbian, can be derived from Hebb-like interactions. Our second term can be justified in a similar way, by averaging neighbour interactions of the sort he describes; the 'elastic net' analogy is only intended to give intuitive guidance on the way the model works. The resulting equation has the advantage of a cleaner mathematical form which can be analysed in detail⁷. Empirically, it gives results similar to his and other self-organizing models, and in our hands is certainly no less computationally effective.

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1. Durbin, R. & Mitchison, G. *Nature* **343**, 644–647 (1990).
2. von der Malsburg, C. *Kybernetik* **14**, 85–100 (1973).
3. Amari, S.-I. *Bull. Math. Biol.* **42**, 339–364 (1980).
4. Kohonen, T. *Bio. Cybern.* **43**, 59–69 (1982).
5. Kohonen, T. *Self-Organization and Associative Memory* 3rd edn (Springer, Heidelberg, 1989).
6. Durbin, R.M. & Willshaw, D. *Nature* **326**, 689–691 (1987).
7. Durbin, R.M., Szepeski, R. & Yuille, A. *Neural Computation* **1**, 348–358 (1989).

DURBIN AND MITCHISON REPLY—As Kohonen points out, self-organizing models have previously been used to simulate cortical maps. We did not intend to suggest the contrary, and we did, in fact, cite

1. Lubin, D., Frederick, J.E., Booth, C.R., Lucas, T. & Neuschuler, D. *Geophys. res. Lett.* **16**, 783–785 (1989).
2. Calkin, J. & Thordardottir, T. *Nature* **283**, 563–566 (1980).
3. El-Sayed, S.Z. *Proc. 5th SCAR Symp. Antarctic Biol.* Hobart (1988).
4. Trodahl, H.J. & Buckley, R.G. *Science* **245**, 194–195 (1989).
5. Trodahl, H.J., Buckley, R.G. & Brown, S. *Appl. Optics* **26**, 3005–3011 (1987).
6. Buckley, R.G. & Trodahl, H.J. *Cold Reg. Sci. Techn.* **14**, 201–204 (1987).
7. Rivkin, R.B., Putt, M., Alexander, S.P., Meritt, D. & Gaudet, L. *Mar. Biol.* **101**, 273–283 (1989).
8. Frederick, J.E. & Snell, H.E. *Science* **241**, 438–440 (1988).

One man and his lab

John Stachel

Lawrence and His Laboratory. A History of the Lawrence Berkeley Laboratory. Vol. 1. By John L. Heilbron and Robert W. Seidel. University of California Press: 1989. Pp. 586. \$29.95.

AN UNDERSTANDING of just how high-energy physics reached its present state of apparently limitless demands on the world's physical and financial resources — as well as on the human resources of many physicists — can well begin with a close scrutiny of “the rise of Big Science as it was born in the Rad Lab” (Martin Kamen, *Radiant Science, Dark Politics*). Those of us who are not technological determinists want to get a sense of how the

if Lawrence “himself had not uncovered much new about the nucleus”, his “notable contribution” was “the cyclotron laboratory” (p. 489). As Livingston, one of the first and most notable of the boys — and one of the first to break free of Lawrence and his lab — put it: “His optimistic and inspirational attitude was what convinced me it was worth working on” (p. 486).

Yet Lawrence himself was changed by

as an experimentalist who had staked his whole career on the building of ever bigger and more elaborately instrumented cyclotrons to become the intimate of university administrators, foundation nabobs and influential industrialists. His induction into the Bohemian club, a retreat for the rich and powerful of San Francisco and their university confrères, fairly early in his career at Berkeley can be taken as an early indication of his success. The award of almost one-and-a-half million dollars by the Rockefeller Foundation and the trustees of the University of California in 1939 marked the culmination of his efforts: “The munificent grant represented many things: dollars to be sure, but also the affection, respect and confidence in which Lawrence's fellow physicists and prominent men of business held him” (p. 482).

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Ernest O. Lawrence holding the first particle accelerator and right, the advanced light source (a synchrotron radiation facility) under construction at the Lawrence Berkeley Laboratory, completion date 1991.

nuclear physicists of the 1930s, through cooperation and conflict with their fellows and with the rest of their social milieu, created the first generation of particle accelerators; and how the result in turn helped to shape the lives and deeds of succeeding generations of particle physicists. We want to get a sense of the alternatives that were faced, the crucial choices that were made — and why — and of potentialities left unexplored as a consequence of these decisions. Heilbron and Seidel have in large measure succeeded in fulfilling these hopes in the first volume of a projected trilogy, which carries the story to 1941, the year of America's entry into World War II.

We watch Lawrence himself pick up the basic idea for the cyclotron, which had previously occurred to many people, and develop it into a working machine, using his enthusiasm, energy and considerable skills as an organizer to build up a remarkable group of doctoral students and post-doctoral co-workers — his “boys” in the typical male-bonding language they all used. Heilbron and Seidel emphasize that,

the course of his own success. The process of reaching for ever-higher energies and ever-bigger machines seems to have become an end in itself for him, an imperative only loosely coupled to uses to which the beams emerging from his machines could be put. “Lawrence sets goals expressible not in terms of progress in physics, but in terms of increases in decimals” (p. 480). While Lawrence and his brother John, a physician who worked on medical applications of the newly available particle beams, were undoubtedly sincere in their efforts to benefit humanity (“It means a great deal more to civilization . . . to find a new radiation or a new substance that will cure disease than it would to discover a new supernova”, Ernest said in 1940), there was just as undoubtedly an element of manipulation in the way these prospects were dangled before prospective donors — and the general public — as a means of obtaining the financial support that became more and more necessary to the realization of Lawrence's ever more ambitious dreams. He was forced by the logic of his position

For the first time, funding for nuclear physics began to rival that for an older American mania for the super in science, big telescopes; the parallel with the 200-inch telescope was explicit in the funding hype for the cyclotron — “the Palomar of the vanishing small”. By this time, almost all the main ingredients for contemporary big science were in one place, except one: large-scale financial support from the federal government. As the authors indicate in the last chapter, World War II was to provide that ingredient.

The change in Lawrence was not without its costs to the laboratory and to the man. A veteran of the lab in the 1930s, Morton Kamen, reports that Lawrence “was almost always on the road by 1939. The early magic of the Rad Lab had largely vanished”, to be replaced by “a new, less personal ambience, unpleasant in its demands for aggrandizement”. Another ingredient had been added to the recipe for a modern high-energy physics facility. Kamen connects Lawrence's “untimely death at the age of fifty-seven of [chronic] ulcerative colitis” with the in-

rence Berkeley Laboratory

flexible demands that he came to make on his family, his lab and himself. There are few hints of this darker side of the Lawrence story in the present volume, although we may anticipate more in the sequel.

Lawrence's ambitions were not only intensive — the biggest machine possible — but also extensive: "There should be a cyclotron lab in every university center" he said in 1938. He generously exported the know-how — and the people who knew how — to physics centres in the United States and abroad. By 1940, "there were 22 cyclotrons completed or under construction in the United States" (p. 308), hardly enough to satisfy Karl K. Darrow, the Polonius of American physics, who opined: "The country may need a thousand cyclotrons" (p. 311).

One gets a sense of lost potentialities for other modes of development of nuclear physics from the initial resistance to the spread of the cyclotron by the best of the British and finest of the French nuclear physicists. Lawrence was often reproached for careless work and unjustified claims in the early years of the lab, such as his notorious argument for the supposed instability of the deuteron. Heilbron and Seidel make a rather harsh assessment: "The frequency with which the laboratory had to retract published results had declined since Lawrence had gone full time into fund raising and administration" (p. 385). In any case, it is certainly a "disagreeable fact that no major discovery had yet been made [by 1940] in any cyclotron laboratory".

The great discoveries in nuclear physics in the 1930s were made by small groups, getting the most out of small accelerators, careful chemical analyses and free-of-charge cosmic rays. No wonder Rutherford and Chadwick, Cockcroft and Joliot-Curie initially resisted the call from California. In another preview of post-war things to come, the first foreign converts to the Berkeley gospel were the Japanese, based on "a conviction on the part of government and industry that excellence in Western science was essential to Japan's place in the sun" (p. 317).

The need to move to higher energies than conventional methods could reach impressed itself on British, French and German savants in the late 1930s; by this time, the initial quirks that had made cyclotronics more of a dark art than a working technology had been resolved, and Lawrence's machine stood ready as the only technique able to meet the need. Something else went along with these exports: "The fascination with hardware and the subordination of the individual to the group that characterized Berkeley by the late 1930s were to spread from the accelerator laboratories to other parts of physics and from the United States to the rest of the world" (p. 351).

Heilbron and Seidel, with Bruce Wheaton, have elsewhere described the story of Lawrence and his laboratory as "a strong interaction between science and society" ("Lawrence Berkeley Laboratory 1931–81" *CERN Courier*, October 1981). Unfortunately, the social context they depict is limited largely to Lawrence's relationships with the powers-that-be. Theirs is a history from the top down, which is certainly an important part of the story. As Raymond Williams observed in another context: "A need which corresponds to the priorities of the real decision-making groups will, obviously, more quickly attract the investment of resources and the official permission, approval or encouragement on which a working technology . . . depends."

But if one is to understand the choices Lawrence made in the 1930s, as compared with those made by Berkeley's other rising physics superstar J. Robert Oppenheimer (the often-made contrast between the two is also made in this book), one must tell some of the history of the 1930s from the bottom up. It was a time of severe economic crisis in the United States, but also a time of great hope. President Roosevelt spoke of "one third of a nation ill-housed, ill-clad, ill-nourished", and of the need to curb the power of the trusts if the country was to be put back on its feet. A wave of protest by the unemployed and of trade-union organization by the employed swept the nation, cresting in California. To understand the California of the time one must evoke not only the Federal Telegraph Company and the Research Corporation, as do the authors, but also the general strike in San Francisco and the efforts to organize migrant farm workers, immortalized in John Steinbeck's *Grapes of Wrath*.

The impact of such events and struggles, which did not leave their own plight unaffected, moved a considerable number of US scientists into political action in broad sympathy with the growing movement to organize labour in factory and field — and laboratory. "Suddenly, prominent scientists were in the forefront of both the antifascist and social reform movements . . . A small though vocal and influential portion of the scientific community became radicalized", reports Peter Kuznick in his recent account of this movement (*Beyond the Laboratory*, University of Chicago Press, 1989). It will not do to say, as do the authors, "Lawrence trusted that the world would muddle through without requiring his attention", while "Oppenheimer did not allow the world to get on without his help" (p. 255). Theirs were political decisions, made in the context of national and international conflicts, the outcome of which were to decide the fate of a generation.

It must be added that the authors' aloof

and ironic style, while adding considerably to the amusement of the reader, does not serve well the goal of a deeper understanding of the personalities of Lawrence or the other central figures in the drama. Instead of patronization, for example, one would have wanted more help in understanding what many of Oppenheimer's students — and students of his career — consider his finest hour as a human being and social figure. Lawrence also made his decisions. His biographer reports that: "He warned his people . . . 'If anyone wants to write letters to editors and that sort of thing, he should get out of science and get a job on a paper'" (H. Childs, *An American Genius*, p. 267). The authors discuss quite frankly Lawrence's anti-semitism, of the conventional mid-western variety typical of his generation, and its repercussions in the lab. They do not mention that: "Though he preferred not to have women in the laboratory at first", presumably because of similar conventional prejudices, "a few were eventually accepted" (Childs). Their own attitude towards women is not unobjectionable: in their text, Lawrence's wife is always "Molly", Lawrence is never "Ernest". Their account of the courtship of lab secretaries (all female) by lab scientists (all male) is reminiscent of anthropological accounts of the exchange of women as a means of male tribal bonding (p. 247).

This long book is not without its *longuers*: much of its length can be attributed to the tendency of the authors to follow their material, however far it leads them from Lawrence and his lab: in some measure, they have written a history of experimental nuclear physics in the 1930s. It would have been helpful if the authors had provided an introductory overview of the entire history of the lab; that they have not done so is surprising since they, together with their former collaborator, Bruce Wheaton, have written two such surveys that carry the story through the seventies (one is cited above; the other, published by the lab, has the same title as this book). Even more surprisingly, neither survey is cited in the extensive bibliography here.

The publisher must be commended for the unusually low price of the book, which certainly justifies its computer-driven look. But something has gone seriously wrong with the index, which is a disaster area. An hour's effort turned up nearly 100 missing page references, and about 40 missing names of people and institutions. A more systematic search of one entry (Szilard) turned up about as many missing references as there are in the index. □

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Serious science

Willem D. Hackmann

Robert Hooke: New Studies. Edited by Michael Hunter and Simon Schaffer. Boydell & Brewer: 1989. Pp. 310. £39.50, \$75.

HOKE — inventor, architect, surveyor of the city of London and man of science — was one of the most brilliant English scientists in the seventeenth century, eclipsed only by Newton. His mechanical genius was surpassed by no one. Yet in many respects he is still an elusive character, undeservedly better known to the history of science fraternity than to the general public. No portrait of him has

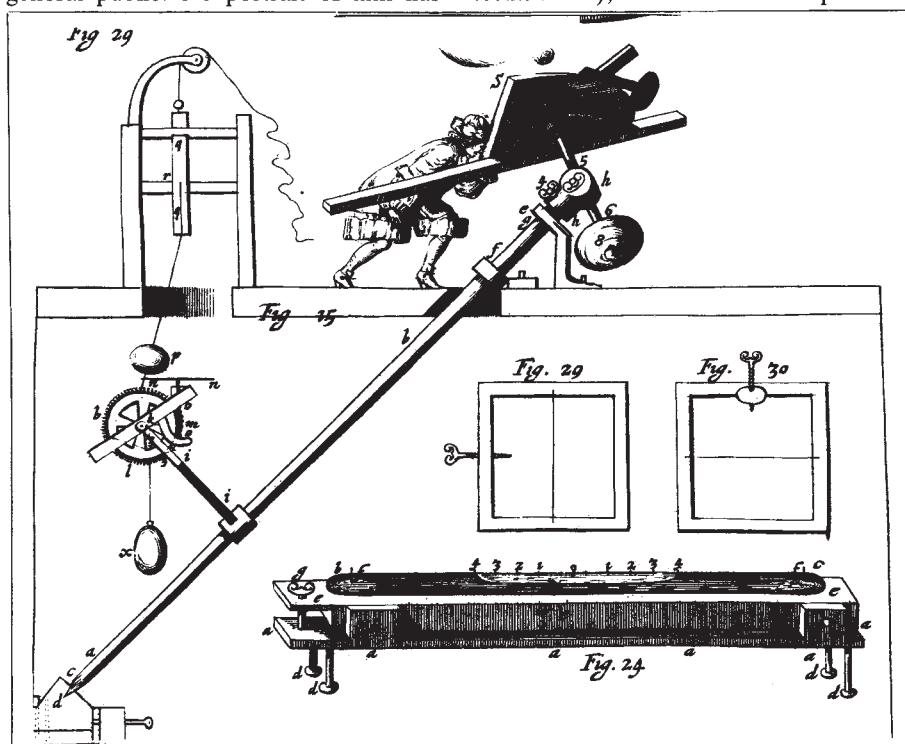
colours (1672) and the priority of the law of gravitation (1686), and with Christiaan Huygens over the spring regulator (1674). He also fell out with the Astronomer Royal John Flamsteed, the Danish astronomer Johannes Hevelius, and with a bevy of Royal Society fellows: Sir John Cutler, Henry Oldenburg, Richard Waller, John Woodward and Hans Sloane, to name but the most prominent. Steve Shapin points out in his perceptive chapter in the present volume that some of these episodes might not have resulted from Hooke's cantankerous nature but rather from the ambivalent place he occupied in the *milieu* of seventeenth century science: not quite a 'gentleman scientist' like Robert Boyle, nor a lowly artisan (Hooke styled himself a 'master of technicians'), but someone in a dependent

to underpin his instrumental view of nature by his scientific method. In the opening chapter, Jim Bennett deals with Hooke's instruments for astronomy and navigation and their common concern with angular measurement. The telescope was scarcely regarded as an astronomical instrument before the application of the eyepiece micrometer, championed by Hooke in the early 1660s. A keystone of Hooke's science was his confidence in the potential for instrumental advance as expressed in his controversy with Hevelius, and the development of his novel equatorial quadrant with its conical pendulum driven clock.

A. D. C. Simpson presents an extraordinary detailed analysis of Hooke's concern with practical optics, highlighting the complicated relations between Hooke and the London optical instrument-making trade, and also between Hooke and his colleagues at the Royal Society. Michael Wright has a particular affinity with Hooke because he is a skilled instrument maker. He not only analyses Hooke's attempts to produce a clock for determining longitude at sea, he actually sets about reconstructing the device in brass. David Oldroyd tackles Hooke's enduring interest in geology.

With John Harwood's essay, the book moves from instrumentation to application of the devices, in this case to the strategic use of pictures produced with the microscope to make the new scientific philosophy generally accepted — what Harwood calls the "visual rhetoric" of Hooke's science. John Hendry examines Hooke's scientific method. Hooke not only used instruments to aid the imperfect senses (calling experimentation the "sixth sense" in scientific endeavour), but also as metaphors for describing natural processes. An important aspect highlighted by Hendry is that Hooke's mechanical philosophy cannot be so easily characterized but contains elements of earlier notions of natural magic. Patri Pugliese describes the real contribution Hooke made to Newton's formulation of his inverse-square law of universal gravitation. It is apparent from the ensuing priority dispute that Hooke never appreciated the full magnitude of the step from his ideas to Newton's achievement.

Light relief is supplied by Lucinda Beier's tale of Hooke's determined search for good health, based on his *Diary*. He collected medical recipes (folk remedies and scientific medications) in the same manner as schoolboys collect stamps, reporting on cures such as the use of the blood of a black cat to cure chilblains. Hooke treated his body like a chemical laboratory, testing out all sorts of medicines, such as different types of 'vomits' to purge the system. He was the great medicine-taker of the seventeenth century. Beier teaches us a great deal



Hooke's equatorial quadrant, with its conical pendulum driving clock. Could the diminutive figure pushing the quadrant be Hooke himself? From Hooke's *Animadversions on the First Part of the Machina Coelestis* (London, 1674).

survived, although we do have a detailed pen drawing by his biographer Richard Waller: "... in person but despicable, being crooked and low in stature, and as he grew older more and more deformed. He was always very pale and lean, and latterly nothing but skin and bone, with a meagre aspect, his eyes grey and full, with a sharp, ingenious look whilst younger. ... He went stooping and very fast, having but a light body to carry, and a great deal of spirits and activity, especially in his youth. ... His temper was melancholy, mistrustful, and jealous, which more increased upon him with his years."

Hooke became notorious in his lifetime for the many bitter controversies and priority disputes with fellow scientists, notably with Newton, over the theory of

position, an employee who had to survive on patronage. It may not be insignificant that there is no record of him ever having crossed swords with that great patron of science, Robert Boyle, with whom Hooke started his scientific career and who obtained for him the post of Curator of Experiments at the Royal Society. As Shapin concludes, Hooke's pattern of behaviour was not considered exceptional among contemporary tradesmen who had to live from the product of their wits.

This multi-authored volume on different aspects of Hooke yet again illustrates the versatility of the man's genius. What emerges are two fundamental strands of Hooke's work: his enthusiasm for mechanisms (what Richard Waller called "his first and last Mistress"), and his need

about contemporary medical practices and attitudes.

What could have been a somewhat dispersive book is ably held together by the introduction. And as its conclusion is a transcript of an hitherto unpublished list of Hooke's possessions at his death discovered in the Public Record Office. Among his dilapidated chattels a chest was found containing £8000 in new and old money, gold and silver. Richard Waller was moved to "a melancholy Reflexion" of how much good this could have done "for the promoting of Learning" if Hooke had only left a will. Instead the money passed on to an illiterate distant relative. Even so, Hooke emerges from this book as the figure that turned the Royal Society into a serious scientific institution. □

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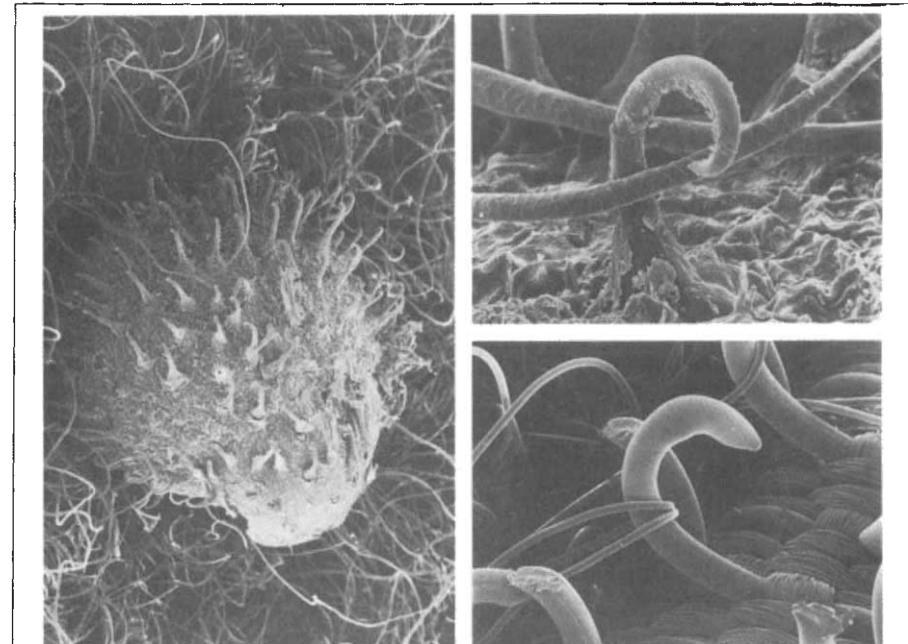
Tribal tribute

Len Goodwin

Laughing Death. The Untold Story of Kuru. By Vincent Zigas. *Humana*: 1990. Pp. 315. \$24.95, £29.50.

VINCENT Zigas, who died two years ago, was born in Germany. He had served as a medical officer in Hitler's army and in 1950, in search of a new life, took Australian citizenship and was posted as a bush doctor to the highlands of New Guinea. He fell deeply in love with the island — its steep mountains, hot, wet forests and, above all, its stone-age people — this is his second autobiographical book about his "green dwelling". Conditions when he arrived were primitive; disease and death were all around. Zigas struggled to understand the native languages, prejudices, taboos and intricate ways of thought and behaviour so that he could improve health and hygiene.

When he went to work in the Okapa district among the Fore people, a distinctive tribe with its own traditions and cannibalistic rituals, Zigas became intrigued with kuru, a neurological disorder attributed to sorcery. Women were more commonly affected than men; the condition struck suddenly, advanced inexorably, causing tremor, ataxia and inane laughter, and was always fatal in about a year. It was not impossible that the strong local belief in sorcery could have caused hysterical behaviour patterns of this kind. As Zigas says, "the native lives in a world in which there is normally very little difference between reality and unreality . . . belief in magic and the supernatural provides ideal soil for the growth of acute suggestibility". But he was not satisfied; he suspected an infectious cause and pleaded with the Australian authorities



Imitating nature — the fruit of the goosegrass (left) hooked onto a woollen sweater provides the model for Velcro, a tough, manufactured nylon 'hook and loop' fastener. The top right micrograph depicts a goosegrass hook with a single snared wool fibre. Bottom right, the synthetic equivalent, a Velcro hook woven into a fine, flexible nylon base material. Micrographs reproduced from the new edition of *Under the Microscope* by J. Burgess, M. Marten and R. Taylor. Cambridge University Press, £12.95, \$19.95.

to back up his investigations. Because of his special relationship with the people, who liked and trusted him, he was able to collect patients for visiting clinicians to examine and blood, urine and post mortem samples for Macfarlane Burnet's scientists in Melbourne.

Nothing much came of Zigas's studies until a visiting virologist from the United States, Carleton Gajdusek, also became fascinated by kuru, avidly collecting case-histories, data and samples for analysis. According to Zigas, who had a big chip on his shoulder about being a 'naturalized' Australian and bossed by those who had never worked in the field and who despised the natives, Gajdusek's activity was resented by the Australian medical hierarchy. The establishment tried to bar Gajdusek and, in the mistaken belief that kuru was a genetic disease, to isolate the Fore people and prevent their movement to other parts of the country. Fortunately, this plan did not succeed.

The suspicion that kuru might be caused by an infectious agent was reinforced by the discovery that scrapie, a disease with similar neurological effects and pathology affecting sheep, could be transmitted to other animals and was caused by a 'slow' virus. Eventually, Gajdusek succeeded in transmitting kuru from human brains to chimpanzees and was awarded a Nobel prize. Transmission in the field was shown to be associated with cannibalism, the women of the tribe preparing the brains of the dead for the ritual feast probably becoming infected through abrasions of the skin.

With the spread of education and the end of cannibalism, kuru has now almost disappeared. But it was the first of the human encephalopathies shown to be transmissible, and this opened the field for the investigation of a whole series of unconventional, very resistant, non-immunogenic viruses and their relation to spongy degenerative diseases of the central nervous system. The lesson taught by kuru, human immunodeficiency virus and, most recently, bovine spongiform encephalopathy seems to be that we transgress the old rules of behaviour at our peril.

This is not a book for the squeamish — bloody stumps alive with maggots, dying infants, rotting corpses, faeces, pus and every kind of secretion and emission are enthusiastically described. Zigas also tells the story of how, on one occasion, he narrowly missed having a kuru brain cooked and served for his own dinner.

Gajdusek could not have succeeded without him. In an affectionate tribute in the foreword to the book, Gajdusek likens Zigas to a Don Quixote or Danny Kaye in appearance, speech, movements and actions. He disagrees with many of Zigas's 'facts' and judgements, and his "uncertain verdict" on the book is that "he has written the most remarkable work in existence of abstract expressionistic ironical parody, his own style of biography" — and that sounds fair. □

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Late Cenozoic uplift of mountain ranges and global climate change: chicken or egg?

Peter Molnar & Philip England

The high altitudes of most mountain ranges have commonly been ascribed to late Cenozoic uplift, without reference to when crustal thickening and other tectonic processes occurred. Deep incision and recent denudation of these mountain ranges, abundant late Cenozoic coarse sediment near them, and palaeobotanical evidence for warmer climates, where high mountain climates today are relatively cold, have traditionally been interpreted as evidence for recent uplift. An alternative cause of these phenomena is late Cenozoic global climate change: towards lower temperatures, increased alpine glaciation, a stormier climate, and perturbations to humidity, vegetative cover and precipitation.

NOT long after continental glaciation had been recognized, Dana¹ linked it to a late Cenozoic rise of mountain ranges. Over the next 100 years, several eminent Earth scientists²⁻⁷ supported and developed this idea, but in all cases the main logical link remained the temporal correlation of mountain building with the onset of glaciation. Then, when geologists discovered more than one Pleistocene glaciation, the significance of this correlation temporarily lost favour.

Now, with Pleistocene glaciations recognized as a consequence of variations in the Earth's orbit⁸ superimposed on a long-term global cooling throughout the Cenozoic era, attention has again been directed to the association of this cooling with regional elevation changes^{9,10}. Uplift of large mid-latitude terrains could effect a global cooling by three different physical mechanisms. First, increased elevations at temperate latitudes could extend the duration of winter, and with increased durations of snow cover, the albedo should increase¹¹. Second, the increase in elevation of large regions should profoundly affect the circulation of the atmosphere, and experiments with general circulation models suggest that the presence of high terrains in Central Asia and western North America would lead to lower global temperatures than if these areas were low¹². These experiments also match a number of observed regional climate

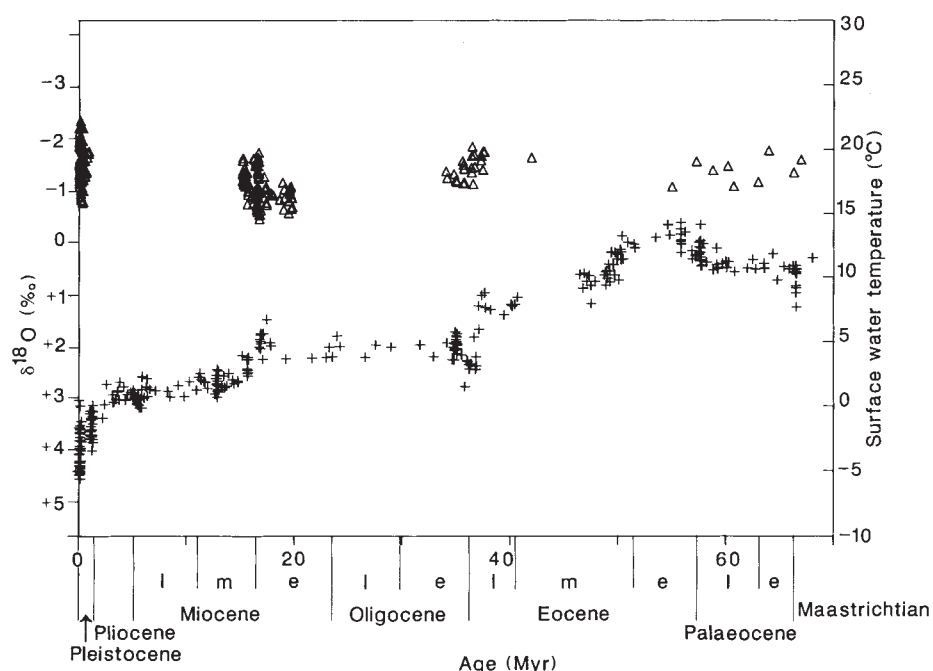
differences that are not obtained in such experiments on landscapes with little or no high terrain¹³. Finally, chemical weathering, enhanced by the increased exposure of minerals eroded rapidly from cold high altitudes and transported to warmer, moister low elevations, would absorb more carbon dioxide from the atmosphere, thus decreasing the natural 'greenhouse effect'¹⁴. In view of the simple logic of these physical mechanisms, and the impressive correlations of calculated with existing regional climate differences¹³, the weak link in the argument relating climate change to late Cenozoic mountain building becomes the inference that mountain ranges and high plateaux rose in late Cenozoic time.

In what follows, we argue that most of the evidence used to infer late Cenozoic uplift could, in large part, be a consequence of the very climate changes that this supposed uplift is thought to have caused. Undoubtedly, some regions are higher today than they were 2 or 10 Myr ago, but many may simply appear to be higher because the geological effects of late Cenozoic climate change could cause the same changes in the landscape and fossil record as regional uplift would.

The Cenozoic climate record

Both palaeobotanical data¹⁵ and oxygen isotopes¹⁶⁻¹⁸ indicate

FIG. 1 Oxygen isotope records (and inferred temperatures) for the past 70 Myr from low-latitude surface waters of the Pacific (Δ) and from ocean deep waters as observed in the South Atlantic (+). Because ocean deep waters are formed at high latitude, the lower curve can be taken as representing the isotope composition of high-latitude surface water. The temperature scale is valid only in the absence of an Antarctic ice sheet—roughly up to mid-Miocene time. Including a correction for the effect of the ice sheet would raise more recent temperatures by $\sim 3^\circ\text{C}$. (Figure courtesy of N. J. Shackleton, modified after ref. 17.)



a monotonic, but not steady, cooling of the Earth over the past 50 Myr. Abrupt decreases in temperature occurred near the Eocene/Oligocene boundary¹⁵⁻²¹ (~36 Myr ago), at ~15 Myr in the Miocene epoch^{16,17,22}, and at ~2.5 Myr near the end of the Pliocene epoch¹⁶⁻¹⁸, when continental glaciation in the Northern Hemisphere began²³. Moreover, the decrease in temperature has been much greater at high than at low latitudes^{15,18,24}. For example, oxygen isotopes from planktonic foraminifera deposited at sub-Antarctic latitudes indicate a decrease in surface water temperatures of ~12 °C since late Eocene time^{16,17}, but those from equatorial latitudes indicate hardly any change in such temperatures^{18,24} (see also Fig. 1). Similarly, a comparison of late Quaternary and present-day elevation ranges of plants growing in the equatorial Andes indicates a temperature difference of ~4.5 °C (ref. 25), but oxygen isotopes from cores in Antarctic ice of the same age imply temperatures 11 °C lower than today²⁶. The resulting increase in latitudinal temperature gradient is important because it promotes an increased transport of water vapour and latent heat from low to high latitudes²⁷, and therefore a stormier climate.

Definitions of uplift

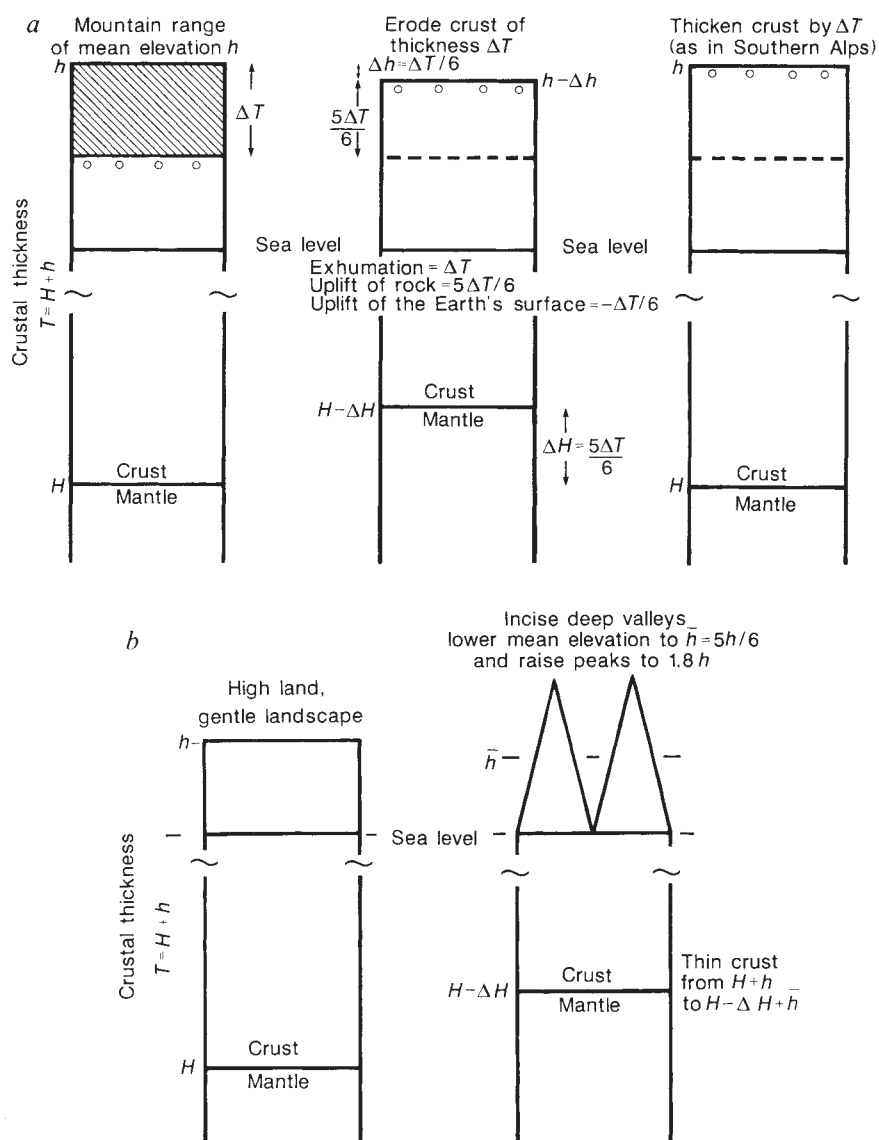
The geological literature is much confused by an inconsistent definition of the word uplift²⁸. Uplift is a vector opposite to the

gravity vector, and a meaningful definition must specify both a reference frame and an object that moves. The most useful reference frames are the geoid (sea level corrected for eustatic changes) and the Earth's surface; the objects that move are either particular rocks or, again, the Earth's surface. In the physical processes linking elevation and climate change¹¹⁻¹³, what matters most is the uplift of the Earth's surface relative to sea level, which is the change in mean elevation. This cannot be measured directly and must be inferred indirectly (see below).

The uplift of rocks relative to the local Earth surface, as measured using geochronology and petrological geobarometers and geothermometers, can be called 'exhumation', and is equal to the thickness of rock removed from the Earth's surface^{28,29}. By isostasy (Archimedes' principle applied to light crust overlying heavier mantle), the removal of a layer of crust of thickness ΔT and density ρ_c , compensated by a crustal root in a mantle of density ρ_m , results in a lowering of the surface of ΔT ($\rho_m - \rho_c$)/ $\rho_m \approx \Delta T/6$ (Fig. 2a). Thus, the isostatic response to rapid erosion of a region of gentle relief and mean elevation h could transform it into one with peaks at heights of $2h$ (or higher), with valleys near sea level, but with a lower mean elevation of only 0.83 h (Fig. 2b).

The uplift of rocks relative to sea level is the sum of the uplift of the Earth's surface and exhumation²⁸. As an example, the heights of marine terraces above sea level (corrected for eustatic

FIG. 2 Simplified crustal sections showing the effects of erosion and crustal thickening on mean elevation, depth of the Moho (the crust/mantle boundary) and uplift of rock. *a*, Imagine a mountain range (left) of mean elevation h , depth to the Moho H and crustal thickness $T = H + h$, and allow a thickness of crust ΔT to be eroded. By isostasy, the mean elevation should decrease by $\Delta h \approx \Delta T/6$, and the underlying rock, including the Moho, should rise by $5\Delta T/6$ (centre). Hence, an exhumation of ΔT yields uplift of rock of $5\Delta T/6$, but a lowering of mean elevation of $\Delta T/6$. Only if, simultaneously, tectonically caused crustal thickening at depth replaces the thickness of crust, ΔT , lost by erosion, will the mean elevation and the depth to the Moho remain constant (right). This is what seems to be happening in the Southern Alps of New Zealand⁵⁰ (see text). *b*, Similarly, if a gentle highland of mean elevation h (left) were deeply incised by rivers that eroded valleys nearly to sea level (right), the mean elevation should drop slightly to about $5h/6$, the remaining rock and Moho would rise by an amount equal to h , and the highest peaks would be much higher than before. In this way, a climatically induced increase in erosion rate can cause exhumation of rocks, and hence the appearance of uplift, with no increase (in fact, with a decrease) in mean elevation.



changes) provide a direct measure of the uplift of the rocks concerned. But the heights of such terraces would equal the change in mean elevation of the terrain only if no material had been removed by erosion. The uplift of such terraces could be caused entirely by tectonic processes, if there were no erosion, or entirely by isostasy, if the uplift of these rocks were merely the isostatic response of the terrain to the removal of material by erosion.

Evidence used to infer recent uplift

We contend that many inferences of recent uplift of mountain ranges are based not on measures of uplift of the Earth's surface, but on estimates either of uplift of rocks or of exhumation²⁸. Furthermore, some of the phenomena used as indicators of uplift could in fact be caused by climate change. An abrupt change in climate, as seems to have occurred at ~15 Myr²², and as clearly occurred at ~2.5 Myr²³, is likely to affect the rate at which the landscape evolves. An increase in alpine glaciation will incise and denude high terrains, and changes in precipitation are likely to affect erosion rates. We suggest that a change towards a cooler and stormier climate could be responsible for the geomorphological evidence ('youthful landscapes') often used to infer recent uplift. Similarly, increased denudation and exhumation should yield an increase in the rate of coarse terrigenous sedimentation, a phenomenon also often attributed to uplift. Finally, global cooling has contributed to the change in mountain flora from warmth-loving species to those characteristic of cooler environments—palaeobotanical evidence that is commonly taken to indicate recent uplift of the fossil material relative to sea level.

Geomorphology. One of the observations commonly used to infer recent uplift is the sharp incision by streams and rivers into gentle surfaces mantled by late Cenozoic sediment. It is a basic tenet of geomorphology that the uplift of a surface with respect to the base level of the streams provides the streams with increased potential energy and steeper gradients, leading to rapid incision of the surface.

Such observations comprise the primary evidence for de Sitter's³⁰ postulation of 2,000 m of Pliocene uplift of the Pyrenees and Alps, and 1,000 m for the High Atlas mountains. From incised erosion surfaces at 1,000–2,000 m on the north flank of the Pyrenees and an extrapolation to 3,000 m near the crest of the range, de Sitter inferred 2,000 m of uplift since the beginning of the Pliocene epoch. Similarly, Trümpy³¹ reported that the Pliocene Alps were 'not more than a hilly tract', whereas now deep valleys near sea level separate ridges with crests rising to 4,000 m. Because the present mean elevation of the Alps is only ~2,000 m³², however, de Sitter's³⁰ inferred uplift of 2,000 m could simply be the isostatic response to the removal of material by the incision of the deep valleys (see Fig. 2b). It is easy to imagine that global cooling enabled alpine glaciers to grow and to excavate deep valleys. We do not suggest that the Alps and Pyrenees underwent no erosion for tens of millions of years before 2.5 Myr, but rather that a change in the processes of erosion and an increased erosion rate reshaped the gentle early Pliocene landscape into jagged relief.

Like those in the Alps and Pyrenees, the major structures of the Rocky Mountains of Colorado, Wyoming and Utah formed in late Cretaceous and early Cenozoic time. Partly because a high erosion surface has been deeply dissected in late Cenozoic time, much of the present elevation of the Rockies is nearly unanimously assigned to the Pliocene and/or Pleistocene epochs^{33–36}. But, as "glacial erosion was the chief cause of the destruction of the Eocene surface in the higher parts of the mountains" (ref. 35, p. 244), here too, climate change should be considered as a contributor to accelerated erosion rates, and the appearance of recent uplift.

Sedimentation. Widespread rapid denudation manifests itself not only by the exhumation of rock, but also by an increased rate of deposition of sediment, near or far from the exhumed

terrain. Thick deposits of late Cenozoic conglomerate surround most steep mountain ranges, and the large cobbles that comprise conglomerate imply transport along steep slopes. Thus, "tectonic activity is commonly held responsible for abrupt coarsening of alluvial fan sequences"³⁷. Potter³⁸ used this argument to infer late Tertiary uplift of the southern Appalachians. Another of the arguments for recent uplift of the Rocky Mountains derives from the presence of thick late Cenozoic conglomerate near them^{33,34,39}. Uplift of one block with respect to another can cause steep gradients, but, as Blackstone³³ noted for one basin in the Rockies, climate change also can affect the potential for streams to transport large cobbles. As Frostick and Reid³⁷ pointed out, infrequent but large storms can produce the abrupt coarsening of conglomerate stratigraphy that is commonly attributed to tectonic processes.

Accelerated denudation is also revealed by sediment accumulation far from mountain ranges. Accumulation rates of pelagic sediment in the Atlantic, Indian and Pacific oceans in late Cenozoic time exceeded those for the rest of that era⁴⁰, with a four- to fivefold increase⁴¹ in the rate of terrigenous deposition since 5 Myr. Like Donnelly⁴², we suspect that the rapid increase in sediment accumulation throughout the world is a consequence of climate change.

The deposition rate since 2 Myr in the Gulf of Mexico, and in particular in the Mississippi delta, has been roughly four times that for the previous 60 Myr (see Fig. 3), and Hay *et al.*⁴³ argued that most of this material has come from the Rocky Mountains. Using constant quantitative relations among elevation differences, rates of sediment transport, base level to erosion, and an assumed history of eustatic sea-level change, they calculated a large late Cenozoic uplift of the Rocky Mountains from this thick Quaternary sediment. Because sediment transport rates also depend on climate^{44–46}, however, a different relationship between sediment transport and elevation for late Cenozoic and earlier times would yield a different, perhaps negligible, inferred Quaternary uplift of the Rockies.

The rapid denudation of the Southern Alps of New Zealand, where a wealth of observations indicate rapid uplift of rock with respect to sea level^{47–49}, illustrates the relationship of uplift of rock to both denudation and tectonics. Adams⁵⁰ showed that the regional erosion rate, determined both from suspended sediment in streams and from sediment accumulations around the island, is comparable to the rate of uplift of rock. Thus, the isostatic response to the removal of material must be a major cause of the present uplift of the marine terraces. To see this, remember that for each 1 km of material removed from the range, the isostatic response is a lowering of the mean elevation by only ~0.17 km (Fig. 2). Thus, although in the steady state that Adams⁵⁰ deduced, tectonic processes seem to maintain a supply of rock to be eroded (by thickening the crust), nevertheless, if tectonic processes stopped, five-sixths of the uplift of rock would still occur. Oblique convergence between the Pacific and Australian plates^{51,52} has occurred in the region of the Southern Alps since 10–15 Myr⁵⁰, but virtually all of the sediment in the basins surrounding the Southern Alps seems to be younger than 2.5 ± 0.5 Myr. Thus, the recent increase in the rates of sedimentation and erosion correlates well with the onset of 'Pleistocene' glaciation, at least as dated in the Northern Hemisphere²³, but not with any resolvable change in plate motion^{51,52}.

Palaeobotany. The main cause of the enormous variation in vegetation over the Earth is the variation in climate. Thus, to use palaeobotany as a palaeobarometer, one must first use it as an indicator of palaeoclimate and then relate that climate to an inference of what the climate would have been in the same region at sea level.

One palaeobotanical approach used to infer palaeoclimates exploits qualitative relationships between assemblages of different taxa and the climates that characterize their present environments⁵³. One assigns the fossil plants 'nearest living relatives', and then finds a present-day environment that includes

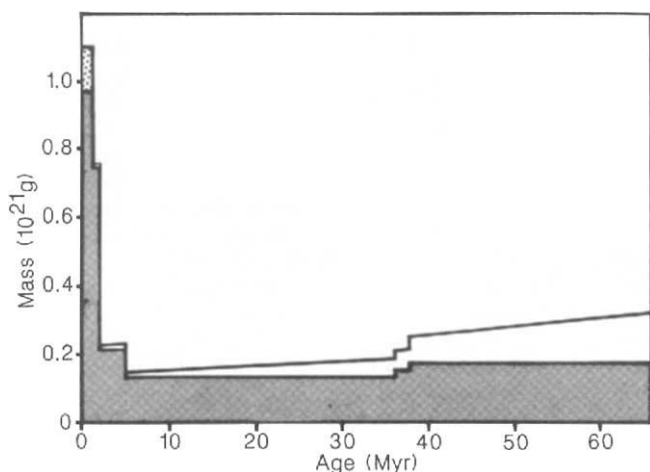


FIG. 3 Mass of Cenozoic sediment in the northwestern Gulf of Mexico and its drainage basin, between the Rocky Mountains and the Appalachians, as a function of the age of the sediment (from ref. 43). The blackened area shows existing sediment, minus an estimate by Hay *et al.* of the sediment transported by the Laurentide ice sheets from the Canadian shield in the past 1–2 Myr and deposited in this drainage basin. The line above shows their estimate of the sediment that was deposited and subsequently removed. Note that the huge increase in sedimentation at ~2 Myr correlates with the abrupt onset of widespread glaciation in the Northern Hemisphere²³.

as many of these relatives as possible. From the characteristics of that environment, one infers a likely palaeoclimate of the region⁵³. The assignment of a nearest living relative is likely to be easiest for the most recent geological past; for Miocene and older times, a substantial fraction of the fossil species are extinct, and the conditions in which many of them lived must have been different from those of their descendants. For example, Axelrod⁵⁴ found that most of the nearest living relatives of fossil taxa from the Eocene Copper River formation in northeastern Nevada grow in either of two types of forest: coastal redwood forests, where elevations range from sea level to ~150 m, and fir-hemlock forests in the Sierra Nevada, at elevations of 1,400–2,400 m. Clearly, assigning a palaeo-elevation (or palaeoclimate) to such a fossil assemblage from present-day equivalents requires an element of subjectivity that makes the method qualitative at best⁵⁵.

Moreover, sometimes the taxonomic classification is based on parts of a plant, such as pollen, that can be relatively insensitive to climate change. Fossil pollen may have fallen from trees whose leaves and wood have since evolved to be adapted to a different climate, while the pollen remains similar. As an extreme analogy, imagine finding late Quaternary tusks in the Siberian Arctic, matching them to the nearest living relative, the elephant, and concluding that because elephants now live in hot, dry savannas at low latitudes, the present North Pole also lay at tropical latitudes in the late Quaternary era. Tusks are not adaptations to climate.

A second palaeobotanical approach to palaeoclimate ignores, insofar as is possible, the taxonomy of the fossil plants and simply uses what is known of how plants adapt to different climates. Using trees from eastern Asia, Wolfe⁵⁶ showed that the percentage of species having leaves with smooth, continuous margins ('whole margins') varies linearly with mean annual temperature, with a scatter of only 2 °C. The application of physiognomic characteristics to palaeoclimates is not without limitations⁵⁶, but climate changes inferred from the physiognomy of fossil plants have been corroborated. From leaf shapes, Wolfe^{15,19,20} deduced an abrupt drop in temperature of several degrees in only a couple of million years near the Eocene/Oligocene boundary. Oxygen isotopes from the sub-Antarctic region near New Zealand later corroborated this rapid decrease in both surface and bottom water temperatures^{16,21}. Using nearest living relatives, however, Axelrod and Bailey⁵⁷ inferred only a gradual change.

Virtually all attempts to use palaeobotany to infer palaeo-elevations have relied on taxonomy, not physiognomy. Moreover, many studies, such as those reporting recent uplift of the Tibetan plateau or the Himalayas^{58–61}, have ignored global climate change. Given that most of the Earth cooled during the late Cenozoic era^{15–18}, it is not surprising that palaeobotanical

observations make it seem that mountain ranges have risen recently. For an average lapse rate of ~6 °C km⁻¹, a secular change in temperature of 6–9 °C since 5–15 Myr^{16–18} could yield an erroneous inference of 1,000–1,500 m of uplift. Moreover, because environmental lapse rates tend to be lower than free-air lapse rates⁶², such a change in mean annual temperature could lead to a larger error in inferred uplift. Thus, although both the Himalayas and Tibet rose in Cenozoic time, the palaeoclimatic inferences of an accelerating rise throughout Cenozoic time^{58–61} (see Fig. 4) are likely to be exaggerated, if not simply false²⁸.

The Rocky Mountains provide another example of exaggerated, palaeobotanically determined, late Cenozoic uplift. MacGinitie⁶³ noted that many of the species from the early Oligocene Florissant flora in central Colorado could not be matched with present-day equivalents from any single, well defined forest; nevertheless, he inferred a relatively warm mean annual temperature of 18 °C and a palaeo-elevation of less than 915 m. The evidence for warm climates where early Eocene (Laramide) deformation had created mountain ranges is a common argument for low palaeo-elevations^{39,64,65}, and the palaeo-elevation of Florissant has provided a base level for estimates of subsequent uplift of the whole of the Rockies and the Great Plains⁶⁵. Recall, however, that the late Eocene/early Oligocene climate was very warm—warmer than it has been since then^{15–18}.

Using the physiognomy of the fossil leaves of the Florissant assemblage, Meyer⁶⁶ inferred a mean annual temperature of 14 °C, not greatly different from MacGinitie's 18 °C. By comparing this with the likely warm early Oligocene sea-level temperature at the same latitude, however, and using an appropriate lapse rate and a 200-m difference between early Oligocene and present sea level, Meyer⁶⁶ inferred a palaeo-elevation of 2,450 m. Even if a smaller sea-level difference were used, this would be indistinguishable from the present elevation of 2,600 m. He also inferred a high palaeo-elevation of 2,800–3,500 m for the nearby Marshall Pass flora of roughly the same age, presently at 2,835–3,080 m. Moreover, from the physiognomic characteristics of the late Oligocene Creede flora, at 2,680 m in central Colorado, Wolfe and Schorn⁶⁷ inferred a mean annual temperature of 0–2.5 °C, which is virtually identical to the present mean annual temperature of 1.9 °C. Ironically, because Wolfe and Schorn⁶⁷ were prejudiced by the prevailing opinion that the Rockies rose only in late Cenozoic time, they suggested that the Creede flora were deposited in a basin where cold air could be trapped. Thus, Meyer's⁶⁶ and Wolfe and Schorn's⁶⁷ analyses, based on physiognomic characteristics of fossil plants and on differences in present and past climates, show that differences between past and present taxa should not be taken as evidence of low elevations in the western United States in mid-Cenozoic time and of a subsequent recent increase in the mean elevation of the Rockies.

Climate change and the appearance of uplift

The arguments above are intended to show that most estimates of late Cenozoic uplift of mountain ranges are exaggerated, at least if changes in mean elevation define uplift, and to suggest that much of the evidence used to infer recent uplift is a consequence of climate change. Most palaeobotanical inferences of recent uplift of the Earth's surface ignore global cooling, which biases these estimates upward. Inferences of changes in mean elevation from elevated geomorphic surfaces, increased denudation or increased sedimentation overlook the parts played by erosion, which depends on climate⁴⁴⁻⁴⁶, and by isostatic rebound. Isostatic compensation of material removed by erosion will elevate the remaining terrain, but the uplift of ridges and peaks with respect to sea level does not necessarily signify an increase in mean elevation. Our attribution of the rapid erosion of mountain ranges in late Cenozoic time to climate change must remain a hypothesis, given that climate change can be very different in different places¹³, that different types of local climate change can increase erosion rates⁴⁴⁻⁴⁶, and that it is difficult to distinguish a local climate change due to global processes from one due to local uplift. Nevertheless, the same difficulties face the geologist trying to show that the recent rapid incision of a major mountain range is due to uplift, instead of global climate change.

The correlations of global climate change with the phenomena used to infer uplift offer some support for the idea that climate change is responsible for these phenomena. The physiognomy of Oligocene fossil plants from the Rocky Mountains^{63,66,67} is consistent with the climates that would be expected at present elevations, if known global climate changes¹⁵⁻¹⁸ are allowed for, but with lower elevations if that global cooling is ignored. Moreover, much of the deep incision of the erosion surface across the Rocky Mountains was due to glaciation³⁵. In fact, most major mountain ranges thought to have risen in late Cenozoic time have been glaciated, and glaciation increased greatly as the Earth became cooler. The onset of rapid sedimentation in New Zealand at ~2.5 Myr⁵⁰, due to rapid erosion of the Southern Alps, correlates with the onset of late Cenozoic cooling and glaciation²³, but not with any obvious change in tectonics^{51,52}. Finally, late Cenozoic uplift has been inferred for mountain ranges throughout the world, yet globally synchronous changes in plate motions, if they have occurred, have been small. Climate change, on the other hand, has been a global phenomenon.

Although the physical mechanisms by which global climate change might affect erosion rates could be different in different regions, several plausible processes are likely to have enhanced erosion. At relatively high altitudes or high latitudes, alpine glaciation is likely to be the dominant process of erosion, and global cooling surely enhanced glaciation. In the absence of glaciation, the principal 'agents of erosion and transportation

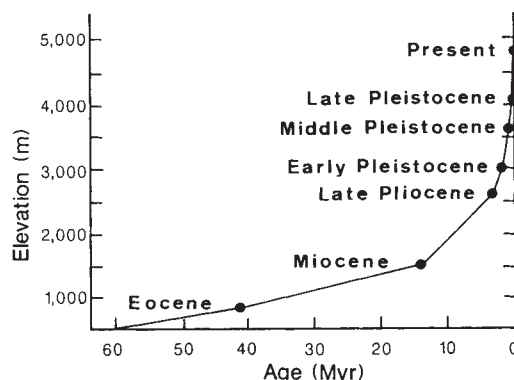
are the impact of raindrops and the surface flow of water'⁴⁶, but variations in precipitation can affect erosion in such extreme ways that one cannot assume that more precipitation would lead to more erosion, or vice versa. In some climates, increased precipitation can increase erosion, but in others, a decrease in precipitation can increase erosion, by reducing the amount of protective vegetation⁶⁸. Whether global cooling led to increased or decreased precipitation is difficult to decide (see below) and likely to vary regionally.

The atmosphere is a thermal engine, which absorbs the Sun's energy in the tropics and transports it upwards and polewards as both sensible heat and latent heat in water vapour. Precipitation in middle latitudes is strongly affected by the ability of the atmosphere to transport humid air from the tropics to cooler higher latitudes. This transport is accomplished primarily by large-scale eddies generated by baroclinic instability²⁷, the same instability that makes the climate stormy. Two aspects of the temperature field affect the rate of transport: the strong temperature dependence of the saturated mixing ratio of vapour and dry air, and the latitudinal temperature gradient. The mixing ratio is proportional to $\exp(-L/RT) = \exp(-5,400/T)$, where L is the latent heat of vaporization, R is the dry-air gas constant, and T is temperature in degrees Kelvin²⁷. The strong dependence of the mixing ratio on temperature makes cooler air much drier than warm air, implying that global cooling would lead to drier climates and less precipitation. The rate of transport, however, is also proportional to the square of the latitudinal temperature gradient. Thus, the inferred change from ~0.25 to 0.5 K per degree of latitude since early Cenozoic time¹⁵ would yield a fourfold increase in latitudinal transport of moisture, but a decrease in mid-latitude temperature of 10 K would yield only a 1.8-fold decrease in water vapour transported. This kind of calculation, which ignores the reduced evaporation at middle latitudes at lower temperatures, is too imprecise to allow a definitive statement about changes in precipitation during Cenozoic time, but it emphasizes that global cooling would not necessarily reduce precipitation. More importantly, the increased latitudinal temperature gradient should have enhanced baroclinic instability, making the climate stormier. Annual erosion rates in many areas are dominated by a few storms^{44,46}; thus, even if global cooling did lead to a drying, as some general circulation model runs suggest⁶⁹, the increased storminess could have increased net erosion.

Climate change and uplift: chicken and egg?

The temporal correlation of late Cenozoic glaciation with phenomena commonly ascribed to recent uplift is inescapable, but assigning the ultimate cause of late Cenozoic climate change to late Cenozoic uplift arising from tectonic processes is a hypothesis that we reject. Instead, we have suggested here that

FIG. 4 Inferred history of uplift of the Tibetan Plateau and the Himalayas, based on palaeobotanical data largely from the north slope of the Himalayas. (Redrawn from ref. 60.) Fossil species whose nearest living relatives grow at relatively low elevations and in relatively warm climates were used to infer lower past elevations, but no account was taken of global cooling, as illustrated in Fig. 1. We show this as an example of inferred uplift, but we doubt that it is precise, or that it applies to all of the Tibetan plateau.



the phenomena commonly used to infer recent uplift are a consequence of climate change.

This is not to dismiss entirely the processes linking elevation and climate described by Birchfield, Kutzbach, Raymo, Ruddiman and Weertman⁹⁻¹⁴; indeed, there is evidence for their operation in a longer-term correlation (if only crude) of climate change with the growth of high terrain in the past 50 Myr¹⁰. There was no Tibetan plateau in late Cretaceous time, when marine limestones were being deposited in the region⁷⁰. Moreover, because the present tectonic activity in Asia seems to be due to the collision of India with Eurasia in Cenozoic time, the high mean elevation of much of the rest of eastern Asia was also probably acquired during Cenozoic time. Thus, the gradual cooling over the past 50 Myr might be the consequence of the tectonic processes that built Tibet, the Himalayas and other high terrains in Asia.

It follows that changes in mean elevation, climate change and the phenomena commonly used to infer uplift are coupled to one another. Although the physical mechanisms linking climate change to uplift should apply most strongly to regions where mean elevation has increased, they could also operate where crests of ranges have risen in response to erosion and isostatic rebound. This raises the possibility that climate change, weathering, erosion and isostatic rebound might interact in a system of

positive feedback. Suppose increased weathering and erosion could enhance a negative 'greenhouse effect'¹⁴; then, if climate change accelerated erosion and weathering, the associated withdrawal of carbon dioxide from the atmosphere might cause further global cooling. Increased rates of erosion and isostatic rebound would alter the distribution of elevations by making the crests of ranges higher than before. The prolonged duration of winter and snow cover in such higher areas might lead to further reductions in temperature¹¹. In addition, mountain ranges with high crests and deep valleys will perturb atmospheric circulation more strongly than those with the same mean elevation but lower crests. Thus, if the rise of crests of mountain ranges were to perturb the climate in such a way as to enhance erosion, isostatic rebound would cause a further rise, and further perturbations to the climate. By such feedback mechanisms, the gradual regional uplift of the Earth's surface in Asia, due to Cenozoic tectonics, might have led to an accelerating climate change and the illusion of accelerated uplift of mountain ranges throughout the world. □

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- Dana, J. D. *Am. J. Sci.* **22**, 305-334 (1856).
- Lyell, C. *Principles of Geology* (Murray, London, 1875).
- Le Conte, J. *Elements of Geology* (Appleton, New York, 1886).
- Ramsay, W. *Geol. Mag.* **61**, 152-163 (1924).
- Umbgrove, J. H. F. *The Pulse of the Earth* (Nijhoff, the Hague, 1947).
- Holmes, A. *Principles of Physical Geology* (Ronald, New York, 1965).
- Hamilton, W. *Met. Monogr.* **8**, 128-133 (1968).
- Hays, J. D., Imbrie, J. & Shackleton, N. J. *Science* **194**, 1121-1132 (1976).
- Ruddiman, W. F. & Raymo, M. E. *Phil. Trans. R. Soc. B* **331**, 411-430 (1988).
- Ruddiman, W. F., Prell, W. L. & Raymo, M. E. *J. geophys. Res.* **94**, 18379-18391 (1989).
- Birchfield, G. E. & Weertman, J. *Science* **219**, 284-285 (1983).
- Kutzbach, J. E., Guetter, P. J., Ruddiman, W. F. & Prell, W. L. *J. geophys. Res.* **94**, 18393-18407 (1989).
- Ruddiman, W. F. & Kutzbach, J. E. *J. geophys. Res.* **94**, 18409-18427 (1989).
- Raymo, M. E., Ruddiman, W. F. & Froelich, P. N. *Geology* **16**, 649-653 (1988).
- Wolfe, J. A. *Am. J. Sci.* **66**, 694-703 (1978).
- Shackleton, N. J. & Kennett, J. P. in *Init. Rep. DSDP Leg 29*, 743-755 (1975).
- Shackleton, N. J. in *Fossils and Climate* (ed. Brencley, P.) 27-34 (Wiley, London, 1984).
- Savin, S. M. A. *Rev. Earth planet. Sci.* **5**, 319-355 (1977).
- Wolfe, J. A. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* **9**, 27-57 (1971).
- Wolfe, J. A. & Hopkins, D. M. in *Tertiary Correlations and Climatic Changes in the Pacific* (ed. Hatai, K.) 67-76 (Sasaki, Sendai, 1967).
- Keigwin, L. D. *Nature* **287**, 722-725 (1980).
- Woodruff, F., Savin, S. M. & Douglas, R. G. *Science* **212**, 665-668 (1981).
- Shackleton, N. J. *et al. Nature* **307**, 620-623 (1984).
- Savin, S. M., Douglas, R. G. & Stehli, F. G. *Geol. Soc. Am. Bull.* **86**, 1499-1510 (1975).
- Liu, K.-B. & Colinvaux, P. A. *Nature* **381**, 556-557 (1985).
- Jouzel, J. *et al. Nature* **329**, 403-408 (1987).
- Stone, P. H. in *Climate Processes and Climate Sensitivity*, *Geophys. Monogr.* 29 (eds Hansen, J. E. & Takahashi, T.) 6-17 (Am. geophys. Un., Washington, DC, 1984).
- England, P. & Molnar, P. *Geology* (in the press).
- Clark, S. P. & Jäger, E. *Am. J. Sci.* **267**, 1143-1160 (1969).
- de Sitter, L. U. *Am. J. Sci.* **250**, 297-307 (1952).
- Trumpy, R. *Geol. Soc. Am. Bull.* **71**, 843-908 (1960).
- Lyon-Caen, H. & Molnar, P. *Geophys. J. int.* **99**, 19-32 (1989).
- Blackstone, D. L. *Geol. Soc. Am. Mem.* **144**, 249-279 (1975).
- Keefer, W. R. *Geol. Surv. prof. Pap. U.S.* 495-D (1970).
- Scott, G. R. *Geol. Soc. Am. Mem.* **144**, 227-248 (1975).
- Tweto, O. *Geol. Soc. Am. Mem.* **144**, 1-44 (1975).
- Frostick, L. E. & Reid, I. J. *geol. Soc. Lond.* **146**, 527-538 (1989).
- Potter, P. E. *J. Geol.* **63**, 115-132 (1955).
- Love, J. D. *Geol. Surv. prof. Pap. U.S.* 495-C (1970).
- Davies, T. A., Hay, W. W., Southam, J. R. & Worsey, T. R. *Science* **197**, 53-55 (1977).
- Hay, W. W., Sloan, J. L. & Wold, C. N. *J. geophys. Res.* **93**, 14933-14940 (1988).
- Donnelly, T. W. *Geology* **10**, 451-454 (1982).
- Hay, W. W., Shaw, C. A. & Wold, C. N. *Geol. Rdsch.* **78**, 207-242 (1989).
- Fournier, M. F. *Climat et l'érosion: la relation entre l'érosion du sol par l'eau et les précipitations atmosphériques* (Presses Universitaires de France, Paris, 1960).
- Wilson, L. *Am. J. Sci.* **B273**, 335-349 (1973).
- Jansson, M. B. *Land Erosion by Water in Different Climates* (Uppsala University, 1982).
- Bull, W. B. & Cooper, A. F. *Science* **234**, 1225-1228 (1986).
- Cooper, A. F. & Bishop, D. G. *Bull. R. Soc. N.Z.* **18**, 35-43 (1979).
- Wellman, H. W. *Bull. R. Soc. N.Z.* **18**, 13-20 (1979).
- Adams, J. *Geol. Soc. Am. Bull. Part II*, **91**, 1-114 (1980).
- Walcott, R. I. *Geophys. J. R. astr. Soc.* **52**, 137-164 (1978).
- Stock, J. & Molnar, P. *Nature* **325**, 495-499 (1987).
- Axelrod, D. I. *Paleobotanist* **14**, 144-177 (1965).
- Axelrod, D. I. *Univ. Calif. Publ. Geol. Sci.* Vol. 59 (1966).
- MacGinitie, H. D. *Univ. Calif. Publ. Geol. Sci.* Vol. 35, 67-158 (1966).
- Wolfe, J. A. *Geol. Surv. prof. Pap. U.S.* 1106 (1979).
- Axelrod, D. I. & Bailey, H. P. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **6**, 163-195 (1969).
- Guo, Shuang-xing in *Geological and Ecological Studies of Qinghai-Xizang Plateau*. Vol. 1, 201-206 (Science Press, Beijing, 1981).
- Hsü, J. (Xu Ren) *Paleobotanist* **25**, 131-145 (1978).
- Xu Ren in *Geological and Ecological Studies of Qinghai-Xizang Plateau*. Vol. 1, 139-144 (Science Press, Beijing, 1981).
- Mercier, J.-L., Armijo, R., Tapponnier, P., Carey-Gailhardis, E. & Tonglin, H. *Tectonics* **6**, 275-304 (1987).
- Price, L. W. *Mountains and Man* (University of California Press, Berkeley, 1981).
- MacGinitie, H. D. *Fossil plants from the Florissant beds, Colorado*, *Carnegie Inst. Washington, Publ.* 599 (1953).
- Axelrod, D. I. & Bailey, H. P. *Paleobiology* **2**, 235-254 (1976).
- Trimble, D. E. *Mountain Geologist* **17**, 59-69 (1980).
- Meyer, H. W. thesis, Univ. Calif. Berkeley (1986).
- Wolfe, J. A. & Schorn, H. E. *Paleobiology* **15**, 180-198 (1989).
- Hall, S. A. *Geology* **18**, 342-345 (1990).
- Hansen, J. *et al.* in *Climate Processes and Climate Sensitivity*, *Geophys. Monogr.* 29 (eds Hansen, J. E. & Takahashi, T.) (Am. geophys. Un., Washington, DC, 1984).
- Norin, E. *Geological Explorations in Western Tibet* (Tryckeri Aktiebolaget, Thule, Stockholm, 1946).

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The protein encoded by the *Arabidopsis* homeotic gene *agamous* resembles transcription factors

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Mutations in the homeotic gene *agamous* of the plant *Arabidopsis* cause the transformation of the floral sex organs. Cloning and sequence analysis of *agamous* suggest that it encodes a protein with a high degree of sequence similarity to the DNA-binding region of transcription factors from yeast and humans and to the product of a homeotic gene from *Antirrhinum*. The *agamous* gene therefore probably encodes a transcription factor that regulates genes determining stamen and carpel development in wild-type flowers.

ARABIDOPSIS THALIANA is widely used for molecular and genetic studies of many developmental processes in plants^{1,2}. We have focused on the molecular basis of pattern formation in developing *Arabidopsis* flowers. Flower development remains one of the most complex processes unique to plants, and little is known about the molecular mechanisms involved. Flowers develop from a few undifferentiated cells into a structure that has several types of organs occupying precisely defined positions. *Arabidopsis* has typical mustard flowers (Fig. 1a) consisting of four concentric whorls of organs³. The first or outermost whorl consists of four green sepals. Interior to and alternating with the sepals are four white petals which make up the second whorl. The third whorl consists of six stamens, four long and two short, each of which has a filament capped by a pollen-bearing anther. The fourth and innermost whorl consists of two fused carpels capped by a short style and stigmatic papillae.

Many mutations affecting floral morphogenesis have been identified in *Arabidopsis*. Mutations in at least four genes result in homeotic transformations of floral organs^{1,3-7}. In general, these homeotic mutations affect the development of adjacent whorls of floral organs. For example, the *ap2-1* mutation results in the conversion of the sepals into leaf-like organs, and in the conversion of petals into stamen-like organs³. Mutations in the *AP3* (*ap3-1*) or *PI* (*pi-2*) genes result in the conversion of petals into sepals and stamens into carpels. Mutations in the *agamous* (*AG*) gene result in the overall phenotype of a flower within a flower and the absence of stamens and carpels. The six stamens have been replaced in *ag* mutant flowers by six petals, and the carpels have been replaced by a new flower. Thus, *ag* flowers consist of ten petals (four normal and six in the place where stamens are normally found) inside of the four sepals, and inside the petals are again four sepals and ten petals (Fig. 1c). This pattern repeats itself and the resulting flower can consist of more than 70 organs. Other species of plants with similar double

flower phenotypes were recognized as long ago as 2,000 years⁸. The first published report of *Arabidopsis* flowers with an *ag* mutant phenotype was more than a century ago⁹, and another *Arabidopsis* mutant having similar flowers has been described by Conrad¹⁰. The extensively characterized³ mutant allele, *ag-1*, was isolated after ethylmethane sulphonate (EMS) mutagenesis and was first described by Koornneef *et al.*¹¹. The *AG* locus has been mapped to chromosome 4 (ref. 11).

Here we describe the molecular cloning and characterization of the *AG* gene, which was facilitated by a T-DNA insertion mutation¹². The deduced *AG* protein product is similar to transcription factors from humans (SRF) and yeast (MCM1, ARG80), and to the product, DEF A, of a recently isolated homeotic gene from the snapdragon *Antirrhinum majus*.

Insertion mutant of *agamous*

Recently, a method has been developed to tag genes by insertion of the T-DNA from *Agrobacterium tumefaciens*¹³. One of the

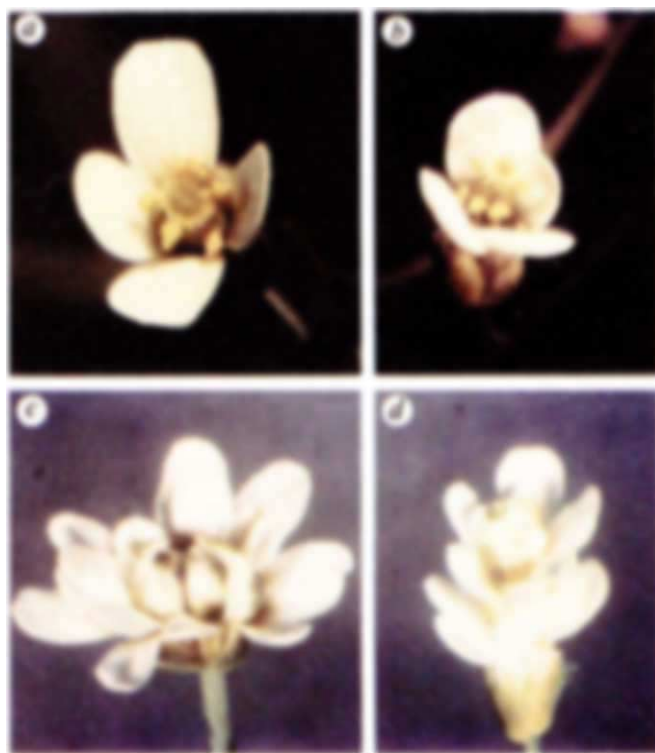


FIG. 1 Photographs of *Arabidopsis* flowers. a, Wild-type; b, *ag-2* plants transformed with pCIT540; c, *ag-1*; d, *ag-2*. Complementation of the *ag-2* mutation was tested by introducing the cosmid pCIT540 into the *Agrobacterium* strain ASE (ref. 26). This strain was used to infect leaf pieces of homozygous *ag-2* mutant plants by standard methods²⁷. A regenerated plant that displayed wild-type flower morphology is shown here.

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sequences in pCIT505 was determined by restriction fragment length polymorphism (RFLP) analysis¹⁴. No crossovers were observed between a pCIT505-linked polymorphism (Fig. 2a) and the *ag-1* mutation in 118 meiotic products scored, indicating that this cloned segment maps less than two centimorgans from the *AG* gene. Taken together, these results suggest that the T-DNA is inserted in or near the *AG* gene and that we have recovered DNA sequences from this region. The pCIT505 plasmid was then used as a probe to screen a cosmid library of wild-type *Arabidopsis* sequences and several clones were isolated and characterized. One of these cosmids, pCIT540 (Fig. 2a), when introduced into the genome of homozygous *ag-2* mutant plants, complemented the mutation and the resulting flowers appeared to be of wild type, with stamens and carpels (Fig. 1b). As the T-DNA insertion has been stably maintained through eight generations, and the introduced sequences restore a wild-type flower phenotype to this stable strain, it seems that this region indeed contains the wild-type *AG* gene.

Nucleotide sequence

Restriction fragments spanning the T-DNA insertion site were used to probe a complementary DNA library prepared from RNA isolated from wild-type *Arabidopsis* flowers. Only one class of cDNA clones spanned the T-DNA insertion site. The nucleotide sequence of several overlapping cDNA clones and the corresponding genomic region was determined (Fig. 2c). These results indicate that this gene contains at least nine exons ranging in size from 42 to 235 bases, and eight introns ranging in size from 84 to 2,985 bases (Fig. 2b). Sequence analysis of the T-DNA-plant junction fragment indicates that the foreign DNA in the *ag-2* allele is inserted in the 2,985-base intron (Fig. 2b).

To confirm that these sequences represent the *AG* gene, genomic fragments were isolated and sequenced from the EMS-generated mutant *ag-1*. The *ag-1* mutation was induced in the same genetic background, Landsberg *erecta*, from which we have isolated and characterized the wild-type sequences. This analysis revealed a single nucleotide change that is presumably responsible for the *ag-1* mutant phenotype. This nucleotide substitution changes the *AG* dinucleotide to *AA* at the acceptor site of the fourth (138-base) intron shown in Fig. 2b.

The deduced protein product contains at least 285 amino-acid residues, although from analysis of the sequences it seems that none of the cDNA clones are of full length. As the deduced open reading frame continues to the 5' end of the longest cDNA, and a translational initiation codon has not been identified, the full-length *AG* protein could be slightly longer. But many attempts with primer extension, anchored polymerase chain reaction and the screening of two different cDNA libraries have been unsuccessful at obtaining longer clones. Nearly all of the isolated cDNAs end within a region of a few nucleotides, suggesting that the RNA may have a secondary structure that prevents extension beyond this region. As the longest cDNAs

	10	20	30	40	50
AG	52	RGKIEIKRIENTTNRQVTFCKRRNGLKKAYELSVLCDAEVALIVFSRGRPLYEYS			
DEF A	3	RGKIQIKRIENOTNRQVTSKRRNGLFKKAHLSVLCDKRVSIIMISSTQKLHEHI			
SRF	144	RVKIMEEIDNKLRYTTFSSKRRCTGIMKKAYELSVLTGTQVLLVASETGHVYTF			
MCM1	18	RRKIEIKFIENKTRRRVTFKRRKGGIMKKAFELSVLTGTQVLLVASETGLVYTF			
ARG80	80	RRKQPIRYIENKTRRRVTFKRRRGGIMKKAYELSVLTGTQVLLVASETGLVYTF			
AG/DEF A		RGKI IKRIEN TNQVTF KRRNGL KKA ELSVLCDA V IV SS L EY	41/56		
AG/MCM1		R KIEIK IEN T R VTF KR G KKA ELSVL V L V S G Y S	32/56		
AG/SRF		R KI I N R TF KR G KAYELS L V L V S G Y	25/56		

FIG. 3 Amino-acid sequence comparison (single-letter code). An alignment is shown for the deduced amino-acid sequences for the gene products from *Arabidopsis thaliana* (AG), *Antirrhinum majus* (DEF A), humans (SRF) and yeast (MCM1, ARG80). Identical residues between AG and each of DEF A, MCM1 and SRF are shown below, as are the fractions of identical amino acids shared by these proteins. Numbers to the left of each sequence indicate the position of the first amino acid shown for each protein.

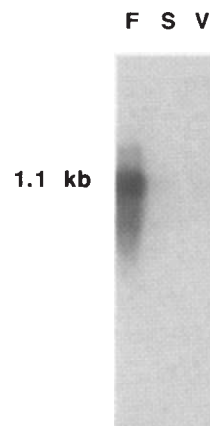


FIG. 4 RNA blot. An autoradiogram is shown for an RNA blot hybridized with ³²P-labelled *AG* cDNA. RNA was from immature floral buds (lane F), floral stems (lane S) and from vegetative tissue (lane V).

METHODS. Poly(A)⁺RNA was isolated from *Arabidopsis thaliana* Landsberg (*er*) as previously described²⁹ and 2 µg each RNA type was size-fractionated on a 1.5% formaldehyde agarose gel, transferred to Hybond N nylon membrane (Amersham), and hybridized with a radiolabelled gel-isolated DNA fragment from the *AG* cDNA, pCIT565. Hybridization was in 50% formamide, 5 × SSC buffer, 10 × Denhardt's buffer, 100 µg ml⁻¹ single-stranded salmon sperm DNA and 10% Dextran sulphate. The filter was washed three times in 2 × SSC, 1% SDS at room temperature and then four times in 0.2 × SSC, 1% SDS at 58 °C. The flower RNA was prepared from a collection of young floral buds before stage 12 (before the flowers open)^{3,20}. Vegetative RNA was obtained by growing plants in a liquid culture for ~18 days before collection; the resulting tissue consisted of roots, stems and leaves. Floral stem RNA was prepared from floral stems with cauline leaves. The vegetative and floral stem RNA preparations also contain a small amount of floral RNA as these tissues contain immature stage-3 flowers²⁰. To prove that RNA was present in the floral stem and vegetative lanes, this RNA blot was also hybridized with a cDNA specific for the *Arabidopsis* G-protein gene *GPA1* (ref. 30); this clone hybridized more strongly to the stem and vegetative RNA (lanes S and V) than to flower RNA.

are of 1,043 base pairs (bp) and the polyadenylated messenger RNA is close to 1.1 kilobases (kb) in length (see below), these cDNAs are of nearly full length. We have recently isolated the two presumed *AG* homologues from the allotetraploid mustard *Brassica napus* and each of these genes contains a translational initiation codon corresponding to the TCG codon at nucleotides 112–114 of the *AG* cDNA sequence (M.F.Y., H.M. and E.M.M., unpublished results). This indicates that all the evolutionarily conserved sequences of the *Arabidopsis* gene have been identified.

The deduced *AG* amino-acid sequence was used to search the database of protein sequences using the program of Lipman and Pearson¹⁵. The *AG* protein shares significant sequence similarity to a class of transcription factors from humans¹⁶ and yeast^{17,18}, and to the product of a recently isolated *Antirrhinum* homeotic gene¹⁹ (see Fig. 3).

RNA analysis

To study the pattern of *AG* gene expression, poly(A)⁺ RNA was isolated from several different tissue types and hybridized with an *AG* cDNA. An RNA from flowers (length ~1.1 kb) hybridized with the *AG* cDNA probe (Fig. 4, lane F). After a much longer exposure, a similarly sized transcript was detected in RNA from inflorescence stems and whole plants before the appearance of the primary inflorescence stem (Fig. 4, lanes S and V), although the intensity of this signal was about 1% of that in flowers. The plant tissue used for these RNA isolations did contain a small number of early developing flowers²⁰, and

it is possible that this low level of signal is the result of RNA in these developing flowers. We estimate the level of AG RNA to be $\sim 10^{-4}$ in poly(A)⁺ RNA of flowering apices, on the basis of both reconstruction experiments and the frequency of cDNA isolation.

To determine if AG expression is organ-specific, *in situ* hybridizations were performed. Sections of wild-type *Arabidopsis* flowers were prepared and probed with ³⁵S-labelled RNA synthesized from the AG cDNA template (Fig. 5). These results indicate that AG is expressed in stamens and carpels and not (or at much lower levels) in sepals or petals.

Discussion

We have isolated and characterized a flower-specific *Arabidopsis* homeotic gene, AG. The insertion mutant allele, *ag-2*, and the EMS-induced mutant allele, *ag-1*, both display alterations in the genomic region from which the presumed AG RNA is transcribed. The changes in the mutant alleles seem to interfere with normal transcription or splicing.

The deduced sequence of the AG protein shows striking similarity to the sequences of transcription factors from both humans (SRF, ref. 16) and yeast (encoded by *MCM1*, ref. 17, and *ARG80*, ref. 18), and to the deduced amino-acid sequence of a recently isolated homeotic gene (*def A*) from the flowering plant *Antirrhinum majus*¹⁹. In humans, the serum response factor (SRF) is thought to be required for the serum-inducible transcriptional activation of genes such as *c-fos*, a nuclear proto-oncogene of mammals¹⁶. In yeast, the product of the *MCM1* gene (GRM/PRTF) is a transcriptional regulator of mating-type-specific genes²¹. The region shared by these proteins spans ~ 56 amino-acid residues and, allowing for conservative substitutions, AG is $\sim 80\%$ similar in this region to all of these proteins (Fig. 3). This same region of SRF encodes the DNA-binding and dimerization domains^{16,22}. On the basis of this sequence similarity and the *ag* phenotype we propose that the AG protein

is a transcription factor involved in regulating genes that determine stamen and carpel development in wild-type flowers.

Like those of *Arabidopsis*, flowers of *Antirrhinum* consist of four concentric whorls of organs (sepals, petals, stamens and carpels). Mutations in the *Antirrhinum def A* homeotic gene cause the conversion of petals into sepals and stamens into carpels. This phenotype does not resemble that of *ag*, but closely resembles the phenotype of the *ap3* and *pi* mutants of *Arabidopsis*³, suggesting that the *Antirrhinum def A* gene could be the homologue of either the *AP3* or *PI* gene of *Arabidopsis*. The AG gene product displays a high degree of similarity to DEF A in the DNA-binding region (Fig. 3), but not elsewhere. As the phenotypes of *Arabidopsis ag* and *Antirrhinum def A* mutants are very different, it seems likely that these genes function in completely different regulatory steps in flower development. The AG and DEF A proteins are more similar to each other than either is to the SRF, MCM1 and ARG80 proteins (Fig. 3).

We have recently isolated a family of other genes from *Arabidopsis*, some of which are preferentially expressed in flowers, that all share extensive sequence similarity to the DNA-binding regions of the SRF-MCM1 class of transcription factors (H.M., M.F.Y. and E.M.M., unpublished results). This indicates that there is a family of related genes that may act to control many of the steps in organ development in *Arabidopsis* flowers. This situation might be analogous to animal development where specific DNA-binding motifs have been adopted for different regulatory circuits. For example, *Drosophila* homeotic selector genes share the homeobox motif, the *Drosophila* gap genes all encode zinc-finger-type proteins, and genes involved in neurogenesis share the helix-loop-helix motif^{23,24}. The MCM1²¹ and SRF²⁵ gene products interact with other factors in regulating gene expression, and it is therefore possible that both AG and DEF A also interact with other factors to exert their regulatory control; some of these factors may be encoded by other homeotic genes known to exist in *Arabidopsis* and *Antirrhinum*.

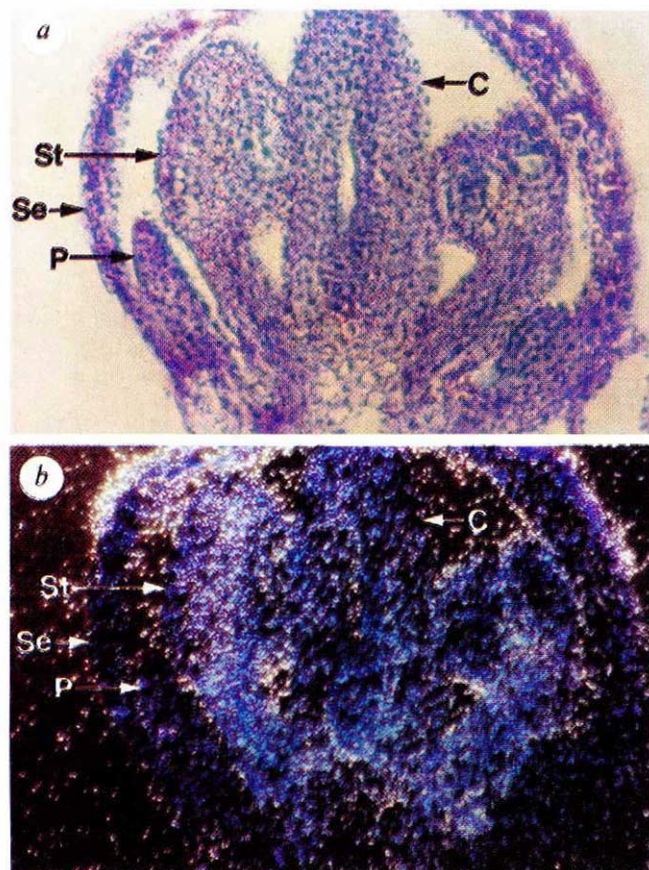


FIG. 5 *In situ* hybridization of AG. To determine the pattern of AG expression, ³⁵S-labelled antisense RNA was synthesized from an AG cDNA template (pCIT565), and hybridized to 8 μ m longitudinal sections of stage-9 *Arabidopsis* flowers^{3,20} as previously described³¹. *a*, Bright field and *b*, dark field. Arrows indicate the position of floral organs including Se (sepals), P (petals), St (stamens) and C (carpels). Hybridization of antisense RNA to the AG mRNA is localized to the positions of stamens and carpels and not to sepals and petals, indicating that it is in these organs that the AG gene is preferentially expressed. The signal over the upper portion of the sepals is observed both with sense and antisense RNA probes and therefore does not represent specific hybridization.

In addition, we used RNA blot analysis to identify the 1.1-kb *AG* mRNA and found it to be preferentially expressed in flowers. *In situ* hybridization experiments show that this RNA is localized to the stamens and carpels of wild-type flowers,

the same organs that are lacking in *ag* mutants. It will be interesting to determine if the pattern of *AG* gene expression is altered in any of the other *Arabidopsis* homeotic flower mutants. □

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1. Meyerowitz, E. M. *Rev. Genet.* **21**, 93–111 (1987).
2. Meyerowitz, E. M. *Cell* **56**, 263–269 (1989).
3. Bowman, J. L., Smyth, D. R. & Meyerowitz, E. M. *The Plant Cell* **1**, 37–52 (1989).
4. Koornneef, M. in *Genetic Maps 1987* 742–745 (Cold Spring Harbor Laboratory, New York, 1987).
5. Pruitt, R. E., Chang, C., Pang, P. P. & Meyerowitz, E. M. in *45th Symposium of the Society for Developmental Biology* (ed. Loomis, W.) 327–338 (Liss, New York, 1987).
6. Komaki, M. K., Okada, K., Nishino, E. & Shimura, Y. *Development* **104**, 195–203 (1988).
7. Kunst, L., Klenz, J. E., Martinez-Zapater, J. & Haughn, G. W. *The Plant Cell* **1**, 1131–1135 (1989).
8. Meyerowitz, E. M., Smyth, D. R. & Bowman, J. L. *Development* **106**, 209–217 (1989).
9. Braun, A. *Gesell. Naturforsch. Freunde z. Berlin* p. 75 (1873).
10. Conrad, D. *Biol. Zentralbl.* **90**, 137–144 (1971).
11. Koornneef, M., de Bruine, J. H. & Goettsch, P. *Arabidopsis Inf. Serv.* **17**, 11–18 (1980).
12. Feldmann, K. A., Marks, M. D., Christianson, M. L. & Quatrano, R. S. *Science* **243**, 1351–1354 (1989).
13. Feldmann, K. A. & Marks, M. D. *Molec. Gen. Genet.* **208**, 1–9 (1987).
14. Chang, C., Bowman, J. L., DeJohn, A. W., Lander, E. S. & Meyerowitz, E. M. *Proc. natn. Acad. Sci. U.S.A.* **85**, 6856–6860 (1988).
15. Lipman, D. J. & Pearson, W. R. *Science* **227**, 1435–1441 (1985).
16. Norman, C., Runswick, M., Pollock, R. & Triesman, R. *Cell* **55**, 989–1003 (1988).
17. Passmore, S., Maine, G. T., Elble, R., Christ, C. & Tye, B. K. *J. molec. Biol.* **204**, 593–606 (1988).
18. Dubois, E., Bercy, J. & Messenguy, F. *Molec. Gen. Genet.* **207**, 142–148 (1987).

19. Sommer, H. *et al. EMBO J.* **9**, 605–613 (1990).
20. Smyth, D. R., Bowman, J. L. & Meyerowitz, E. M. *Plant Cell* **2** (in the press).
21. Herskowitz, I. *Nature* **342**, 749–757 (1990).
22. Hayes, T. E., Kitchen, A. M. & Cochran, B. H. *Proc. natn. Acad. Sci. U.S.A.* **84**, 1272–1276 (1987).
23. Murre, C. *et al. Cell* **58**, 537–544 (1989).
24. Ingham, P. W. *Nature* **335**, 25–34 (1988).
25. Schröter, H., Mueller, C. G. F., Meese, K. & Nordheim, A. *EMBO J.* **9**, 1123–1130 (1990).
26. Fraley, R. T. *et al. Bio/Technology* **3**, 629–635 (1985).
27. Lloyd, A. M. *et al. Science* **234**, 464–466 (1986).
28. Velten, J. & Schell, J. *Nucleic Acids Res.* **13**, 6981–6998 (1985).
29. Crawford, N. M., Campbell, W. H. & Davis, R. W. *Proc. natn. Acad. Sci. U.S.A.* **83**, 8073–8076 (1986).
30. Ma, H., Yanofsky, M. F. & Meyerowitz, E. M. *Proc. natn. Acad. Sci. U.S.A.* **87**, 3821–3825 (1990).
31. Barker, S. J., Harada, J. J. & Goldberg, R. B. *Proc. natn. Acad. Sci. U.S.A.* **85**, 458–462 (1988).

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LETTERS TO NATURE

Detectability of γ -rays from clumps of dark matter

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IF the dark matter in our Galaxy is made up of weakly interacting massive particles (WIMPs) with masses of the order of several GeV (for example, photinos or Higgsinos), γ -rays produced by their annihilation would in principle be observable^{1,2}. But the expected flux³ from a smoothly distributed dark matter halo^{4,5} is much smaller than the observed diffuse background⁶, and although narrow lines might be produced, their intensity would be much too low to see with the Gamma Ray Observatory (GRO)^{3,7}. A complementary approach is to consider unique spatial signatures. Numerical simulations of galaxy formation⁸ show that even in the central bulge of the Galaxy, the mean density of the dark matter could be equal to that of the stars. If this were so, GRO could see the Galactic Centre as a source of annihilating dark matter^{1,3}. Other lumps formed as part of the hierarchical formation of the Galaxy could also produce sources that would be recognized by the shape of their continuum spectrum^{2,3} and a line feature in sufficiently bright sources^{3,7}. Even Geminga^{9,10}, the second strongest source of γ -rays at energies greater than 50 MeV, could be annihilating dark matter.

Lumps clearly formed during the hierarchical formation of the Galaxy. Clusters of galaxies are large-scale versions of this process, several per cent of their mass is contained in galaxian lumps with masses of $\sim 10^{-4} M_{\text{cluster}}$ and density contrasts of $\sim 10^2$. As shown below the density contrast needs to be higher for the lumps to survive in the Galaxy, so I assume that a lower percentage of the mass is in such lumps. These lumps must either be the remnant cores of larger structures or have formed from non-gaussian initial conditions^{11,12}.

The dark matter in the halo has a local density^{4,5} $\rho_{h,0} \approx 10^{-2} M_{\odot} \text{pc}^{-3}$. The distance to the nearest lump is $D_{\text{near}} \approx (M_{\text{cl}}/\eta\rho_{h,0})^{1/3}$, where η is the fraction of the dark matter in lumps with cluster masses, M_{cl} , of $M_6 \times 10^6 M_{\odot}$. The flux of

γ -rays with energies > 50 MeV observed from this lump is

$$F = 5.8 \times 10^{-11} \text{ cm}^{-2} \text{ s}^{-1} (\sigma V)_{26} \left(\frac{X}{7} \right) m_{\lambda,5}^{-2} M_6^{-2/3} \times \left(\frac{\eta\rho_{h,0}}{10^{-2} M_{\odot} \text{pc}^{-3}} \right)^{2/3} \int_0^{\infty} \rho_{\text{cl}}^2 r^2 dr \quad (1)$$

where $(\sigma V)_{26}$ is the annihilation cross-section in units of $10^{-26} \text{ cm}^3 \text{ s}^{-1}$, $m_{\lambda,5}$ is the WIMP mass in units of 5 GeV and the integral over the density in the lump, ρ_{cl} has units $M_{\odot} \text{pc}^{-3}$. X , the average number of γ -rays with energies > 50 MeV per annihilation, is fixed by e^+e^- collider data¹³ and is $7.1 m_{\lambda,5}^{1/2}$. For the particular case of photinos⁷ with masses > 5 GeV

$$(\sigma V)_{26} \approx 0.1 (\Omega_{\lambda} h_{100}^2)^{-1} m_{\lambda,5}^{-2} \quad (2)$$

where h_{100} is the Hubble constant, H , in units of $100 \text{ km s}^{-1} \text{ Mpc}^{-1}$, and Ω_{λ} is the fraction of the closure density in WIMPs.

I adopt a density profile appropriate for lumps that have undergone mutual collisions and tidal shocking by the disk of the Galaxy¹⁴

$$\rho_{\text{cl}} = \frac{\rho_0}{(1 + r^2/r_c^2)^2}; \quad \int_0^{\infty} \rho_{\text{cl}}^2 r^2 dr = 2.3 \overline{\rho_{1/2}} M_{\text{tot}} \quad (3)$$

where M_{tot} is the total mass, $\overline{\rho_{1/2}}$ is the mean density inside the half-mass radius, and r_c is a core radius.

The density $\overline{\rho_{1/2}}$ constrains the redshift of dissipationless formation for the lumps¹⁵ and determines their ability to survive in the Galaxy. When galaxies form without dissipation, they reach maximum expansion and turn around when their density is a factor of 5.5 greater than the critical density $\rho_{\text{crit}} = 3H^2/8\pi G$, where G is the gravitational constant. If pressure is unimportant, the protogalaxy collapses by a factor of two to become bound and virialized. Hence, the relationship between the redshift of turn-around, z_{turn} and $\overline{\rho_{1/2}}$ is

$$(1 + z_{\text{turn}}) = 43 h_{100}^{-2/3} \left(\frac{\overline{\rho_{1/2}}}{1 M_{\odot} \text{pc}^{-3}} \right)^{1/3}$$

The density $\overline{\rho_{1/2}}$ also determines whether the lumps survive mutual collisions and tidal shocking by the galactic disk. Using

the impulse approximation, one can calculate the timescale for disruption owing to mutual collisions of uniform-density spheres of mass M_1 , radius r and density ρ_1 (see Appendix B of ref. 16). Equating M_1 to M_{cl} and ρ_1 to $\bar{\rho}_{1/2}$ leads to an underestimate of the disruption timescale for the more tightly bound lumps with density profiles given by equation (3). The fractional energy change during a collision with impact parameter $d \gg r$ and relative velocity v is

$$\Delta E/E = \frac{2GM_1^2}{3\pi d^4 v^2 \rho_1} \quad (4)$$

During the age of the Universe t_U the closest encounter will have a typical impact parameter given by $d_{close}^2 = M_1/\pi v t_U \eta \rho_h$, where $\eta \rho_h$ is the density of the colliding objects. Because the energy change is dominated by the closest encounter

$$\Delta E/E = \frac{2\pi G t_U^2 \eta^2 \rho_h^2}{3\rho_1} = \frac{\eta^2 \rho_h^2}{27\rho_{crit}\rho_1} \quad (5)$$

where the Friedmann equations with a cosmological density parameter of unity were used to replace t_U^2 with the critical density of the Universe ρ_{crit} . Adopting $\Delta E/E = 1$ as the critical value for survival versus destruction, the survivors must have densities

$$\rho_1 > \frac{\eta^2 \rho_h^2}{27\rho_{crit}} = 13\eta^2 M_\odot \text{ pc}^{-3} \quad (6)$$

When the density in the lumps satisfies this inequality, the dominant disruption mechanism becomes tidal shocking as they pass through the disk¹⁷. This has a timescale, $t_{shd} = 10^{10} (\bar{\rho}_{1/2}/10 M_\odot \text{ pc}^{-3}) \text{ yr}$. With $\eta_{-2} = (\eta/10^{-2}) = 1$ and $\rho_{10} = (\bar{\rho}_{1/2}/10 M_\odot \text{ pc}^{-3}) > 1$, the lumps survive collisions with one another and the disk. The nearest lump is at a distance $D_{near} \approx 10 \text{ kpc} (M_6/\eta_{-2})^{1/3}$. In the general direction of the Galactic Centre, the flux is that expected from a smooth model of the halo.

The expected flux from the nearest lump is then

$$F = 6.8 \times 10^{-6} \text{ cm}^{-2} \text{ s}^{-1} m_{\lambda,5}^{-7/2} \eta_{-2}^{2/3} M_6^{1/3} \times (\Omega_\lambda h_{100}^2)^{-1} \rho_{10} \left(\frac{\rho_{h,0}}{10^{-2} M_\odot \text{ pc}^{-3}} \right)^{2/3} \quad (7)$$

If z_{turn} were fixed rather than $\bar{\rho}_{1/2}$, there would be no dependence on the Hubble constant. The source brightness falls by a factor of two at a distance $R = 10 \text{ pc} (M_6/\rho_{10})^{1/3}$ from its centre, yielding an angular extent of ~ 3.6 arcmin. The freedom in choosing the factors in the product $m_{\lambda,5}^{-7/2} \eta_{-2}^{2/3} M_6^{1/3} \rho_{10}$ makes it impossible to predict source brightnesses. I have chosen values to yield a model where the brightest sources would appear in the Cos-B catalogue¹⁸.

There are two immediate questions. Are there known lumps of dark matter that are potential sources? And are there any known γ -ray sources that might be identified with such lumps?

The extreme dwarf spheroidal satellites of the Milky Way, Draco and Ursa Minor, are good examples of dark lumps¹⁹. They are too distant to be detected, but their existence is difficult to reconcile with gaussian initial conditions¹¹. Peebles¹² found that the Corona Cloud of galaxies is inconsistent with gaussian initial conditions. There are also two candidate 'dark clusters' with properties similar to those that I have assumed, one²⁰ with a distance of $\sim 3.6 \text{ kpc}$, $M_6 = 0.5$ and $\bar{\rho}_{1/2} \approx 5 M_\odot \text{ pc}^{-3}$ and another²¹ with a distance of $5\text{--}8 \text{ kpc}$, $M_6 = 0.2$ and $\bar{\rho}_{1/2} \approx 3 M_\odot \text{ pc}^{-3}$. Both of these clusters could be detected with GRO. Peebles²² suggested that all globular clusters reside in dissipationless haloes with $M_6 \approx 100$ and $\rho_{10} \leq 1$, making them potential sources for GRO. Finally, I²³ invoked similar lumps so that the disk dark matter²⁴ could be the same as the halo dark matter.

As previously stated, several parameters have been chosen to yield fluxes comparable to the bright sources in the Cos-B catalogue. Among these, Geminga is an excellent candidate. It has an observed flux¹⁰ of $4.8 \times 10^{-6} \text{ cm}^{-2} \text{ s}^{-1}$, making it the

second strongest source of γ -rays with energies $> 50 \text{ MeV}$. Its angular size is $< 0.4^\circ$, the angular resolution of Cos-B. X-ray²⁵ and optical counterparts²⁶ have been tentatively identified with luminosity ratios $L_\gamma/L_X \approx 1,000$ and $L_X/L_{opt} \approx 1,800$. The current models are a binary pair of neutron stars²⁷ at a distance of $< 11 \text{ pc}$ or a young pulsar at a distance of 1 kpc whose radio emission is beamed away and whose synchrotron nebulae is mysteriously absent²⁶. The precise nature of this unusual source and its identification with the optical and X-ray counterparts remain uncertain. Geminga has a galactic latitude of only 4° which might make its association with the halo rather than the disk seem odd. But 9% of the total solid angle is within 5° of the disk, the surveys have concentrated on this region and the action of dynamical friction on lumps draws them toward the plane of the disk²⁸. Nonetheless, some sources should be detected at high galactic latitude if this model is valid. The initial GRO survey with EGRET should accumulate 10^4 counts and determine Geminga's spectrum and spatial extent. The continuum spectrum is uniquely determined by the mass of the particle¹⁻³. It will be possible to observe the narrow lines^{3,7} with greater integration time or stacking of similar sources²⁹. $\square$

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1. Silk, J. & Bloemen, H. *Astrophys. J.* **313**, L47–L50 (1987).
2. Stecker, F. *Phys. Lett. B* **201**, 529–532 (1988).
3. Stecker, F. W. & Tylka, A. J. *Astrophys. J.* **343**, 169–176 (1989).
4. Caldwell, J. R. & Ostriker, J. P. *Astrophys. J.* **251**, 61–87 (1981).
5. Kuijken, K. & Gilmore, G. *Mon. Not. R. astr. Soc.* **239**, 571–603 (1989).
6. Fichtel, B. C., Simpson, G. & Thompson, D. *Astrophys. J.* **222**, 833–849 (1977).
7. Bouquet, A., Salati, P. & Silk, J. *Phys. Rev. D* **40**, 3168–3186 (1989).
8. Carlberg, R. G., Lake, G. & Norman, C. A. *Astrophys. J.* **300**, L1–L4 (1986).
9. Thompson, D. J., Fichtel, C. E., Hartman, R. C., Kniffen, D. A. & Lamb, R. C. *Astrophys. J.* **213**, 252–268 (1977).
10. Bignami, G. F. in *Cosmic Radiation in Contemporary Astrophysics* (ed. Shapiro M. M.) 175–192 (Reidel, Dordrecht, 1986).
11. Lake, G. *Astrophys. J.* (in the press).
12. Peebles, P. J. E. *Astrophys. J.* (in the press).
13. Braunschweig, W. et al. *Z. Phys. C* **45**, 193–208 (1989).
14. Aguilar, L. & White, S. D. M. *Astrophys. J.* **307**, 97–109 (1986).
15. Gott, J. R. A. *Rev. Astr. Astrophys.* **15**, 235–66 (1977).
16. Carr, B. J. & Lacey, C. G. *Astrophys. J.* **316**, 23–35 (1987).
17. Ostriker, J. P., Spitzer, L. & Chevalier, R. A. *Astrophys. J.* **176**, L51–L56 (1972).
18. Hermesen, W. et al. *Nature* **269**, 494–495 (1977).
19. Aaronson, M. & Olszewski, E. in *IAU Symp. 177 Dark Matter in the Universe* (eds Kormendy, J. & Knapp, J.) 153–165 (Reidel, Dordrecht, 1987).
20. Sommer-Larsen, J. & Christensen, P. R. *Mon. Not. R. astr. Soc.* **225**, 499–299 (1988).
21. Doiudis, S. P. & Beers, T. C. *Astrophys. J.* **340**, L57–L60 (1989).
22. Peebles, P. J. E. *Astrophys. J.* **277**, 470–477 (1985).
23. Lake, G. *Astr. J.* **98**, 1554–1556 (1989).
24. Bahcall, J. N. *Astrophys. J.* **287**, 926–944 (1985).
25. Bignami, G. F., Caraveo, P. A. & Lamb, R. C. *Astrophys. J.* **272**, L9–L13 (1983).
26. Halpern, J. & Tytler, D. *Astrophys. J.* **330**, 201–217 (1988).
27. Nulsen, P. & Fabian, A. *Nature* **312**, 48–50 (1988).
28. Quinn, P. & Goodman, J. *Astrophys. J.* **309**, 472–484 (1986).
29. Caillault, J.-P. & Helfand, D. *Astrophys. J.* **289**, 279–299 (1985).

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Free electron density in H I regions as an indicator of ionizing dark matter

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IT has recently been proposed¹ that the free electrons giving rise to the observed dispersion of pulsar signals result mainly from photoionization induced by the decay of dark matter particles in the Galaxy. For reasons related to conservation of energy and momentum the decay photons would have a unique energy in the rest frame of the decaying particles, and in the proposed theory

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this energy is close to 14 eV. A crucial prediction of the theory is that in regions where the dark matter density is approximately constant, the free electron density n_e in opaque H I regions should also be approximately constant, when averaged over several mean free paths for the ionizing photons. The determination of an accurate distance for the pulsar PSR1451-68 (ref. 2), in combination with its known dispersion measure and with previous observations of two other pulsars with accurate distances, leads to a successful test of this prediction.

The dark matter particles concerned are likely to be neutrinos of non-zero rest mass. It is well known that neutrinos of mass ~ 30 eV created by pair production in the Big Bang would possess the critical cosmological density if the Hubble constant is $\sim 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$. Such a mass is now ruled out for electron-type neutrinos but not for μ - and τ -types. In my theory¹ the decaying neutrinos turn out to have a well defined mass m_1 of this order, 27.7 ± 0.5 eV. Such a neutrino would be expected³ to decay into a photon and a neutrino of another type and lower rest mass m_2 . If $m_2 \ll m_1$ the energy of the decay photon would be $\sim \frac{1}{2}m_1$, here $\sim 13.85 \pm 0.25$ eV. Such a photon would just be able to ionize hydrogen.

The flux of these decay photons depends on the concentration n_ν of neutrinos and on the lifetime τ for their decay. The concentration can be deduced from the observed rotation velocity of the Galaxy, and is $n_\nu \approx 5 \times 10^7 \text{ cm}^{-3}$ (ref. 1). For a decay photon energy of ~ 14 eV the interstellar medium would be highly opaque (for a neutral hydrogen density of $\sim 0.1 \text{ cm}^{-3}$ the mean free path is ~ 1 parsec), and the photoionization equilibrium is then simply

$$\frac{n_\nu}{\tau} = \alpha n_e^2 \quad (1)$$

where α is the appropriate recombination coefficient. If this mechanism is to account for the value of n_e implied by observed pulsar dispersion measures, $\sim 0.05 \text{ cm}^{-3}$ in H I regions near the Sun⁴, τ must be $\sim 10^{23}$ s (ref. 1). The standard electroweak model gives⁵ a much larger value of τ , but various non-standard models are under investigation^{5,6} which could give the required value.

Here I am primarily concerned with the value of n_e within a few hundred parsecs of the Sun, but will first mention the implications of equation (1) for more distant regions of the Galaxy. The recombination coefficient is expected to be fairly constant; it depends on the kinetic temperature T of the electrons, approximately as $T^{-1/2}$. Therefore, $n_e \approx T^{1/4}$, a weak dependence. Moreover, in the heating regime of concern, T would not be expected to deviate from 10^4 K by more than a factor of about two in the intercloud medium over large regions of the Galaxy⁷. I can therefore assume that α is constant.

I now consider the variation of n_e with galactocentric radius r and height z above the galactic plane. Following the analysis of the pulsar data by Lyne, Manchester and Taylor³, $n_e \approx 1/(1+r/a)$, where the core radius a is $\sim r_\odot$. Equation (1) then implies that $n_\nu \approx 1/(1+r/a)^2$. This leads to a test of the theory, because the r -dependence of n_ν influences the rotation curve of the Galaxy. In fact, there is good agreement with the observed rotation curve⁹. As regards the dependence of n_e on z , one would expect to find an essentially constant n_e at heights less than the scale height of the neutrino distribution. But this would clearly cease to be true at the height where the total gas density becomes less than the constant value of n_e implied by equation (1). Above this height the gas must cease to be opaque, and the assumption of local photoionization equilibrium would break down. Above this point n_e would be essentially equal to the total gas density. Recent observations of the dispersion measure of pulsars in globular clusters up to 20 kpc above the plane indicate that this transition layer occurs at $z \approx 600$ pc (A. G. Lyne, P. Salucci and D.W.S., manuscript in preparation).

In the region a few hundred parsecs from the Sun, one would expect n_ν to be approximately constant and τ to be exactly

constant, being determined by considerations of elementary-particle physics. Thus n_e should be independent of position in opaque regions near the Sun, when averaged over several mean free paths, that is, over several parsecs. By contrast, if the ionizing photons were produced by the relatively widely separated O or B stars, and were subject to complex and position-dependent absorption effects, one would expect to find that the n_e in H I regions derived from the pulsar dispersion-measure data differ for the line segments to different pulsars.

Two of the pulsars for which the free electron densities are known and for which accurate distances have been determined from parallax measurements, PSR0950+08 and PSR0823+26, were recently discussed by Reynolds⁴. The line segments involved are separated by $\sim 28^\circ$, and do not intersect any known H II regions. The inferred mean values of n_e were 0.023 cm^{-3} and 0.054 cm^{-3} , respectively. Reynolds⁴ pointed out that a correction is necessary for the part of the line of sight that passes through the low-density bubble¹⁰ immediately surrounding the Sun. This bubble contains X-ray emitting gas at a temperature of $\sim 10^6$ K and at a correspondingly low density. The correction is important for the first pulsar and Reynolds⁴ concluded that the gas responsible for its dispersion measure must be confined primarily to the outer 47 pc of the line segment, leading to an electron density in that region of $\sim 0.056 \text{ cm}^{-3}$. By contrast, the correction for the second pulsar is small. Thus essentially the same value of n_e in normal H I regions was derived for both line segments, $\sim 0.05 \text{ cm}^{-3}$. Reynolds also concluded, from a detailed study of the O and B stars near these line segments, that these stars were probably not mainly responsible for the ionization along the segments, but could have contributed ~ 10 –20% of the observed ionization.

Using the newly determined² distance for PSR1451-68 (450 ± 60 pc) and the dispersion measure, the electron density is $0.019 \pm 0.003 \text{ cm}^{-3}$. This value is much smaller than 0.05 cm^{-3} , and correction for the local bubble (the radius of which is < 50 pc in the direction of this pulsar)¹⁰ would not make much of a difference. My hypothesis thus requires another suitably large bubble along the line of sight to this pulsar. The galactic co-ordinates of PSR1451-68 are $l \approx 315^\circ$, $b \approx -8.5^\circ$. This line of sight passes right through loop I, a well-known nearby supernova remnant. The position and geometry of this loop are clearly shown in Fig. 2 of ref. 10. To correct for the presence of this loop, I assume a path length of 300 pc, and adopt $n_e \approx 10^{-2} \text{ cm}^{-3}$ (refs 11, 12) along this segment. This low density is associated with the high temperature of this X-ray emitting region. The contribution to the dispersion measure is therefore $\sim 3 \text{ cm}^{-3} \text{ pc}$. As the observed dispersion measure is $8.6 \text{ cm}^{-3} \text{ pc}$, I conclude that the final ~ 100 pc of path length through a normal H I region¹³ to the pulsar contributes $\sim 5.6 \text{ cm}^{-3} \text{ pc}$. Thus n_e in the section of the path through the normal H I region $\sim 0.056 \text{ cm}^{-3}$, in good agreement with the corresponding quantity for the first two pulsars with well determined distances, and with the prediction of my theory.

The most direct observational test of the hypothesis would be to search for an unidentified line in the background at an energy of ~ 14 eV. This line would be produced by decaying particles in the immediate vicinity of the Sun, so that its strength F can be predicted only in relation to an understanding of the local interstellar medium. This problem is discussed elsewhere¹⁴, where I obtained the estimate $F \approx 2 \times 10^3 \text{ cm}^{-2} \text{ s}^{-1}$.

The necessary narrow-band observations in the far ultraviolet have not so far been carried out. The upper limit for the flux of a line at ~ 14 eV derived from the broad-band observations of Holberg¹⁵ is $\sim 3 \times 10^4 \text{ cm}^{-2} \text{ s}^{-1}$, which is compatible with my estimate.

An attempt to search for a line in the background in the vicinity of 14 eV is being planned in collaboration with S. Bowyer and R. Stalio. If it is discovered at the flux level indicated here, it would place the hypothesis of decaying dark matter on a firm footing. $\square$

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1. Sciamma, D. W. *Astrophys. J.* (in the press).
2. Bailes, M., Manchester, R. N., Kesteven, M. J., Norris, R. P. & Reynolds, J. E. *Nature* **343**, 240–241 (1990).
3. de Rujula, A. & Glashow, S. L. *Phys. Rev. Lett.* **45**, 942–944 (1980).
4. Reynolds, R. J. *Astrophys. J.* **348**, 153–160 (1990).
5. Melott, A. L., McKay, D. W. & Ralston, J. P. *Astrophys. J.* **324**, L43–L46 (1988).
6. Mohapatra, R. N. *Phys. Lett. B* **201**, 517–524 (1988).
7. Dalgarno, A. & McCray, R. A. *Rev. Astr. Astrophys.* **10**, 375–426 (1972).
8. Lyne, A. G., Manchester, R. N. & Taylor, J. H. *Mon. Not. R. astr. Soc.* **213**, 613–639 (1985).
9. Salucci, P. & Sciamma, D. W. *Mon. Not. R. astr. Soc.* **244**, 9P–11P (1990).
10. Cox, D. P. & Reynolds, R. J. *A. Rev. Astr. Astrophys.* **25**, 303–344 (1987).
11. Iwan, D. *Astrophys. J.* **239**, 316–327 (1980).
12. Nousek, J. A., Fried, P. M., Sanders, W. T. & Kraushaar, W. L. *Astrophys. J.* **258**, 83–95 (1982).
13. Prentice, A. J. R. & ter Haar, D. *Mon. Not. R. astr. Soc.* **146**, 423–444 (1969).
14. Sciamma, D. W. *Mon. Not. R. astr. Soc.* (in the press).
15. Holberg, J. B. *Astrophys. J.* **311**, 969–978 (1986).

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Discovery of two radio pulsars in the globular cluster M15

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WE report the discovery of two radio pulsars, 2127+11B and 2127+11C, in the globular cluster M15 (NGC7078), which also contains the 110-ms pulsar 2127+11A (ref. 1). Although only twenty globular cluster pulsars are known at present, the detection of three pulsars in a single cluster suggests that there might be a large total population of these objects, which would make them powerful probes of the dynamics and evolution of globular clusters. One of the new pulsars, 2127+11C, is in a highly eccentric binary system with an 8-hour period. It is thus similar to the famous PSR1913+16 system², and study of the pulse arrival times can be expected to provide tests of general relativity, including gravitational wave emission. The companion of PSR2127+11C is probably either another neutron star or a massive white dwarf, suggesting that the core of M15 contains a high density of massive stellar remnants.

The initial observations were made using the 305-m Arecibo radio telescope at a central frequency of 430 MHz and a 10-MHz receiver bandwidth. The data were sampled at an effective rate of 1.974 kHz using the Arecibo 40-MHz three-level correlation spectrometer in a manner identical to that used in the discovery of PSR2127+11A (ref. 1). The discovery observations were made on 26 December 1988 starting at 18:51 UT. The pulse search was carried out on the NCUBE/10 (California Institute of Technology), a concurrent computer having 512 central processing units interconnected with hypercube topology. Assuming a dispersion measure of 67.25 pc cm⁻³, the measured value¹ of PSR2127+11A, a de-dispersed time series of 2²⁴ points was formed by supplementing the observed 10.9 million samples with the mean value. The power spectrum was calculated using an implementation of the fast Fourier transform (FFT) algorithm for concurrent computers² and was searched for families of significant harmonic peaks.

Table 1 and Fig. 1 give the parameters and pulse profiles of the three pulsars detected in the 430-MHz observations. PSR2127+11A was clearly detected in our first analysis of the December 1988 data. Further inspection revealed PSR2127+11B with a period of 56 ms, about twice as faint as PSR2127+11A. Subsequent timing measurements spanning 230 days indicate that PSR2127+11B is an isolated pulsar with a period derivative $\dot{P}$ of 9×10^{-18} s s⁻¹, implying a characteristic age of 1×10^8 yr, if the observed $\dot{P}$ is due solely to magnetic braking. If, on the other hand, one assumes that the dominant effect is acceleration due to the local gravitational field, as in the case

of PSR2127+11A, then the magnitude of the acceleration is 5×10^{-8} m s⁻². The errors quoted for PSR2127+11B in Table 1 are three times the formal errors given by the temporal fitting program used in the analysis (TEMPO). The accuracy of these measurements are limited by the coupling of the timing residuals owing to a position error (a sine wave of period one year) and a $\dot{P}$ error (quadratic drift) over the limited observation period of 230 days.

PSR2127+11C was discovered using an algorithm specifically developed for detection of pulsars in binary orbits. The variable Doppler shift due to the orbital acceleration can spread the signal power over multiple frequency bins, resulting in a loss in sensitivity, particularly for fundamentals or harmonics with millisecond periods. In our algorithm, samples were inserted or deleted in the time series to compensate for a presumed acceleration. To reduce the computational demands of the search, we assumed a constant acceleration. A range of accelerations from -19.2 m s⁻² to $+19.2$ m s⁻² was explored using 2²⁴-point transforms, corresponding to the acceleration experienced by a pulsar in a circular orbit with 1-d period and a $1-M_{\odot}$ companion. A series of 2²²-point transforms was also calculated for accelerations up to 203 m s⁻², corresponding to a $1-M_{\odot}$ companion and a 4-h circular orbit. PSR2127+11C, with a period of 30.5 ms, was initially found in a 2²⁴-point transform at an acceleration of -9.5 m s⁻².

Timing measurements were begun to determine the keplerian parameters of the PSR2127+11C orbit. Recording of the raw data stream on magnetic tape allowed us to extract timing information for all three pulsars from the same data. Because of the faintness of PSR2127+11C, an integration time of ≥ 10 minutes was required for detection. This necessitated an acceleration search for each observation to compensate for orbital motion and to obtain the correct apparent pulsar period. The determination of the orbital period was also complicated by the limited duration (3 h) over which the cluster can be observed by the Arecibo telescope. The orbital period was found to be 8.05 h, indicating that it would take a minimum of 6 weeks for a complete cycle of observable orbital phase. In Fig. 2, we display the apparent pulsar period at the midpoint of each observation as a function of the orbital phase for observations taken primarily over an 8-week period. The eccentricity of the orbit is immediately apparent.

Using the velocity curve in Fig. 2 for an initial estimate of the orbit, we analysed the pulse arrival time on 17 observations taken during May 1988. The resulting keplerian parameters, surprisingly similar to those of PSR1913+16 (ref. 2), are given

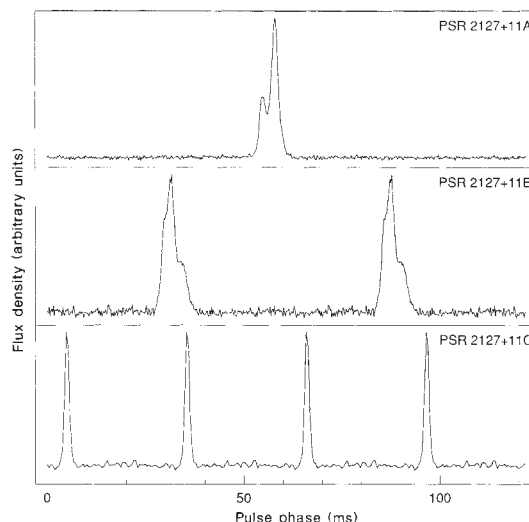


FIG. 1 Average pulse profiles for the three known radio pulsars in globular cluster M15. The time resolution is 500 μ s.

TABLE 1 Pulsar parameters

Timing parameters		2127 + 11A	2127 + 11B	2127 + 11C
Epoch (JD)	T_0	2,447,213.15	2,447,632.9906	2,447,660.9089
Pulse period (ms)	P_0	110.66470954 (1)	56.13303358 (1)	30.529 (1)
Period derivative ($10^{-18} \text{ s s}^{-1}$)	$\dot{P}$	-20 ± 1	8.8 ± 1.1	—
Right ascension (1950.0)*	α_{m15}	$-1.95 \pm 0.15''$	$+3.86 \pm 0.15''$	—
Declination (1950.0)*	δ_{m15}	$+0.6 \pm 0.3''$	$-0.8 \pm 0.6''$	—
Dispersion measure (pc cm^{-3})	DM	67.25 ± 0.05	67.25 ± 1.0	67.25 ± 1.0
Flux density (mJy)	S_{430}	1.7 ± 0.4	1.1 ± 0.4	0.6 ± 0.2
Orbital period (s)	P_b	—	—	$28,969 \pm 1$
Projected semi-major axis (light s)	$a_1 \sin i$	—	—	2.52 ± 0.01
Eccentricity	e	—	—	0.680 ± 0.003
Longitude of periastron (deg)	ω_0	—	—	316.4 ± 1.0
Epoch of periastron (JD)	T_0	—	—	2447632.46720 (5)
Mass function ($M_\odot$)	f	—	—	0.15

Timing parameters for PSR2127 + 11A, B and C. The values given for PSR2127 + 11A are those reported in Wolszczan *et al.*¹ and are consistent with the results of our 430-MHz observations. The values for PSR2127 + 11B are based on 430-MHz observations spanning 230 days. The keplerian orbital parameters for PSR2127 + 11C are based on 17 days of pulse arrival timing. Numbers in parentheses indicate the uncertainty in the last significant figure.

* Positions relative to the centre of globular cluster M15 ($\alpha = 21^h 27^m 33.35^s$, $\delta = 11^\circ 56' 48.8''$)^{1,6}.

in Table 1. If the secondary is another neutron star, the estimated orbital decay time and the advance of periastron will be 2×10^8 yr and 4° yr^{-1} , similar to those of PSR1913 + 16. The PSR2127 + 11C system should thus provide tests of general relativity in the strong-field limit, including gravitational wave emission, as does PSR1913 + 16. The errors quoted in Table 1 are our best estimates of the uncertainty arising from the limited time span of observations. A significantly more accurate measurement of the pulsar parameters, including a precise determination of the advance of periastron, is in progress using approximately nine months of timing observations.

Constraints can be placed on the pulsar and companion masses (M_p and M_c) by determining the mass function f , which depends on the apparent size of the pulsar's orbit and the orbital period. The value of the mass function for the 2127 + 11C system is $f(M_p, M_c) = (M_c \sin i)^3 / (M_p + M_c)^2 = 0.15 M_\odot$. Assuming $M_p = 1.4 M_\odot$ for PSR2127 + 11C, the minimum mass of the companion is $0.94 M_\odot$. For $M_p = M_c = 1.4 M_\odot$, the inclination angle i of the orbital plane to the line of sight would be 49° , close to the median inclination angle of 60° . Given the measured orbital parameters, the distance of closest approach is of the order of $R_\odot$ for inclination angles corresponding to a $1-M_\odot$

companion. A main-sequence companion is therefore ruled out, and the companion is most likely a second neutron star or a massive white dwarf.

The discovery of three pulsars in a single cluster suggests that pulsars are abundant in globular clusters. Kulkarni *et al.*⁴ have quantitatively analysed the results of the major surveys of cluster pulsars and conclude that a typical rich cluster contains about 100 active pulsars, most of which will be undetectable owing to the great distances to the clusters. Assuming a distance of 10 kpc to M15, the radio luminosities of PSR2127 + 11A, B and C are respectively 170 mJy kpc^2 , 110 mJy kpc^2 and 60 mJy kpc^2 . These luminosities are well above the minimum luminosity of 1 mJy kpc^2 for field pulsars. Hence with increased sensitivity, other pulsars ought to be detectable in M15. We are now analysing all our data (>50 h of observations) in an effort to locate fainter pulsars.

The discovery of the binary pulsar, 2127 + 11C strongly suggests an abundance of massive stellar remnants in the core of M15. According to the standard scenario, in some previous phase, PSR2127 + 11C must have been in a binary system with a non-degenerate companion. We rule out 2127 + 11C being a primordial binary because the formation of such systems is inevitably associated with a large systemic motion ($\geq 200 \text{ km s}^{-1}$)⁵⁻⁷ which would have resulted in the expulsion from the cluster. Consequently, the companion is probably the result of a capture interaction. Alternatively, PSR2127 + 11C could have been a solitary spun-up pulsar (like PSR2127 + 11B) that experienced an exchange collision with a binary system. The large observed eccentricity indicates that an exchange may have taken place recently.

Various authors have argued⁸⁻¹⁰ that accretion-induced collapse (AIC) of white dwarfs can produce pulsars. AIC scenarios invoke massive C+O white dwarfs or O-Ne-Mg white dwarfs and thus, even if AIC mechanism were to be operative (see Verbunt *et al.*¹¹ for a discussion of the considerable theoretical and observational difficulties with this mechanism), the discovery of PSR2127 + 11C implies an abundance of these white dwarfs. Such white dwarfs evolve from massive main-sequence stars ($M \leq 8 M_\odot$). Thus in the framework of either the standard or the AIC model we conclude that, in the past, M15 contained many massive stars. To achieve reasonable rates for exchange or capture interactions, the remnants of these massive stars must have aggregated in the core of M15 by mass segregation. Even taking this effect into account, very steep initial mass functions ($\propto M^{-\alpha}$, $\alpha \geq 3.5$) are ruled out for M15 because they predict insufficient numbers of massive white dwarfs and neutron stars. A flatter initial mass function also explains the large abundance of pulsars inferred from other observations⁴. We note, however, that some recent theoretical studies¹² suggest steep initial mass

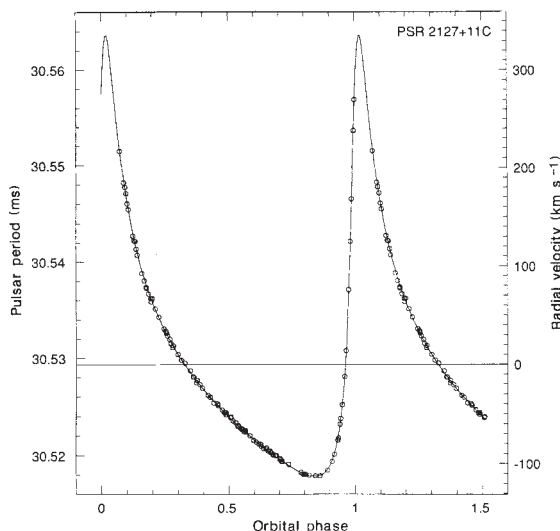


FIG. 2 Velocity profile and observed Doppler-shifted pulse periods as a function of orbital phase for PSR2127 + 11C. The solid line is the apparent pulse period derived from the five keplerian orbital parameters given in Table 1.

functions for most clusters that have survived to the present.

Most tidal-capture binaries (those captured by transfer of kinetic energy to stellar deformation) are expected to be formed with an orbital radius of $\sim R_{\odot}$, and thus an orbital period of a fraction of day. A small fraction (10–30%) are expected to be wide binaries ($P_{\text{orb}} \approx 100$ d) formed by capture of giants. The formation of single pulsars like 2127+11A and B is easily understood as resulting from the breakup of a wide binary by passing stars^{13,14}. Rappaport *et al.*¹⁵ have shown this to be a very effective mechanism for breaking up wide binaries in the especially high-density collapsed core of M15. The tidal interaction model predicts that there should be 3–10 times more compact binaries than isolated or wide-orbit binaries, whereas we detected only one compact binary and two isolated pulsars. Selection effects may have prevented detection of a larger number of short-orbital-period systems in our survey and a quantitative analysis of them will be presented elsewhere. $\square$

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1. Wolszczan, A. *et al.* *Nature* **337**, 531–533 (1989).
2. Fox, G. C. *et al.* *Solving Problems on Concurrent Processors* (Prentice-Hall, Englewood Cliffs, 1988).
3. Taylor, J. H. & Weisberg, J. M. *Astrophys. J.* **253**, 908–920 (1989).
4. Kulkarni, S. R., Narayan, R. & Romani, R. W. *Astrophys. J.* **356**, 174–183 (1990).
5. Cordes, J. M. & Dewey, R. J. *Radio Wave Scattering in the Interstellar Medium* 217–221 (Am. Inst. Phys., New York, 1988).
6. Dewey, R. J. & Cordes, J. M. *Astrophys. J.* **321**, 780–798 (1987).
7. Verbunt, F. & Hut, P. in *The Origin and Evolution of Neutron Stars*, IAU Symp. 125 (eds Helfand, D. J. & Huang, J. H.) 187–197 (Reidel, Dordrecht, 1986).
8. Michel, F. C. *Nature* **329**, 310–312 (1987).
9. Channugam, G. & Brecher, K. *Nature* **329**, 696–698 (1987).
10. Bailyn, C. D. & Grindlay, J. E. *Astrophys. J.* **353**, 159–167 (1990).
11. Verbunt, F., van Paradijs, J. & Lewin, W. G. H. *Mon. Not. R. astr. Soc.* **241**, 51–57 (1989).
12. Chernoff, D. & Weinberg, M. *Astrophys. J.* **351**, 121–156 (1990).
13. Verbunt, F., van den Heuvel, E. P. J., van Paradijs, J. & Rappaport, S. A. *Nature* **329**, 312–314 (1987).
14. Romani, R. W., Kulkarni, S. R. & Blandford, R. D. *Nature* **329**, 309–310 (1987).
15. Rappaport, S., Putney, A. & Verbunt, F. *Astrophys. J.* **345**, 210–221 (1989).
16. Shawl, S. J. & White, R. E. *Astr. J.* **91**, 312–316 (1986).

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Coexistence of two liquid crystalline phases in poly(γ -benzyl- α , L-glutamate) solutions

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IN 1956, Flory¹ proposed a phase diagram for rod-like molecules of the form shown in Fig. 1. Since then, most of the major features of this diagram have been verified experimentally². In particular, the two-phase 'chimney' is now well established. One feature that has not previously been established, however, is the slight maximum (the 'cap') in the phase boundary on the high-concentration side of the chimney. Flory¹ pointed out that this cap should manifest itself as two coexisting liquid-crystalline (anisotropic) phases. Here we provide evidence for bulk phase separation into two such phases in a macroscopic (~ 0.5 g) liquid-crystal sample held in a constant-temperature bath. A distinct boundary can be seen between the two phases, and they are distinguished further under an optical polarizing microscope.

Benzyl alcohol solutions of the synthetic polypeptide poly(γ -benzyl- α , L-glutamate), chosen as a model rod-like molecule, gel at $\sim 60^\circ\text{C}$. Details of sample preparation can be found elsewhere³.

We have previously found⁴ evidence for separation of the gel into two anisotropic phases at $\sim 100^\circ\text{C}$. In that experiment a small sample was heated in a hot stage so surface effects may have been significant and the phases may not have been in equilibrium. Here we immersed small-bore test tubes containing ~ 0.5 g of 15% gel (molecular weight 248,000) in a constant-temperature bath. Macroscopic phase separation (with an easily visible boundary) was achieved at temperatures $\sim 80^\circ\text{C}$ after a day or so. A room-temperature photograph (Fig. 2, left) shows a tube with the two phases clearly visible. (Cooling rapidly to room temperature does not affect the appearance of the phase separation, but the quality of the photograph is better because it is not necessary to photograph through the hot oil.)

Examination of samples of the upper and lower phases in an optical polarizing microscope shows that both are anisotropic. The lower phase is strongly coloured and shows definite 'fingerprinting'⁵ with a periodicity of $\sim 15\ \mu\text{m}$ (Fig. 2, lower right).

The upper phase takes the form of a black and white speckle (Fig. 2, upper right). The two phases are clearly distinct; this is clear evidence for the equilibrium coexistence of two liquid-crystalline phases.

The phase separation takes about a day, that is, the boundary takes about a day to form. Given that the samples consist of 0.5 g of a highly viscous material, it is not surprising that a large-scale separation should take so long to occur. To find the lowest temperature at which the phase separation occurs, samples were heated at a rate of $\sim 1^\circ\text{C}$ per day. The first separation was observed at 71°C and examination of the lower phase showed that it was both coloured and characterized by 'fingerprints'. Previously we saw formation of the coloured phase at higher temperatures, but these results are not necessarily inconsistent because in the first experiment the heating rate was much faster, 2°C min^{-1} , and the sample was much smaller. In the first experiment we observed the coloured phase immediately upon formation whereas in the experiments reported here it is not observed until bulk phase separation has occurred.

Russo and Miller⁶ have seen evidence for the cap in the temperature dependence of fingerprint spacing at fixed concentration. As they reduced the temperature, the fingerprint spacing decreased and then at a certain temperature it began to increase again. From this they inferred that at this temperature the sample passed from the one-phase region to the two-phase cap. They did not actually see the second phase.

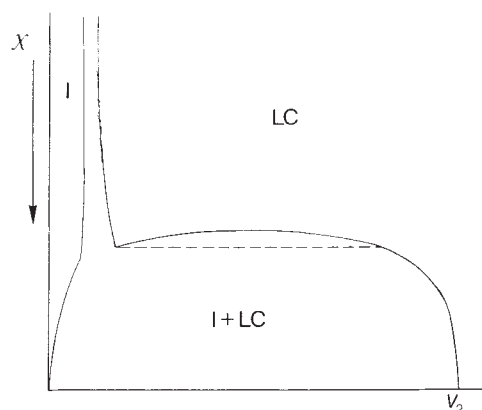
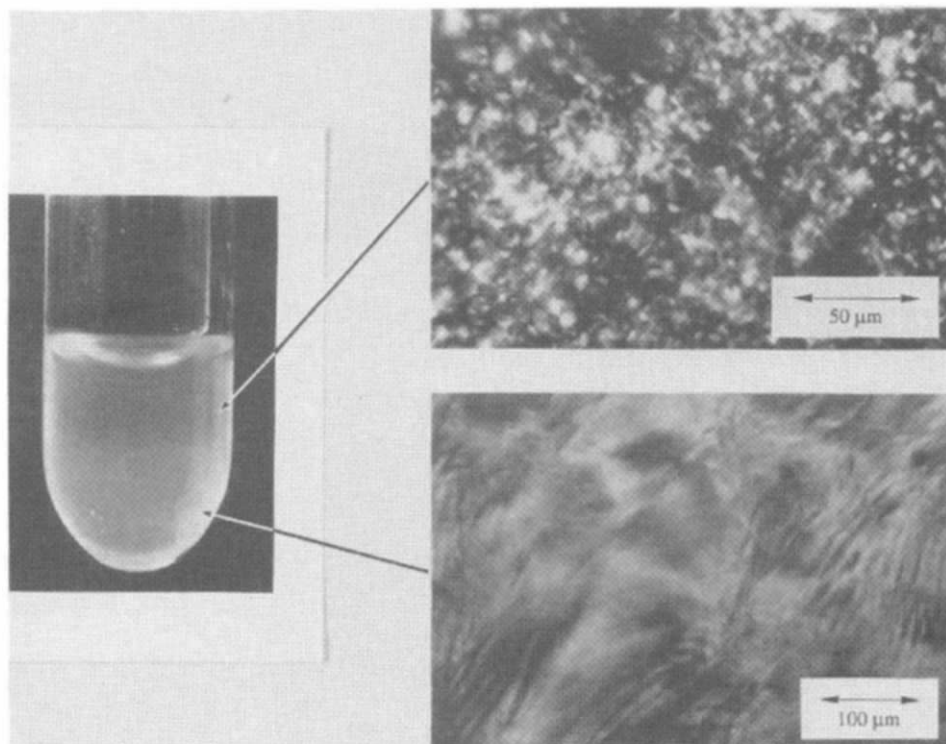


FIG. 1 The phase diagram of rod-like molecules as described by Flory¹. The axes are quality of solvent, χ ($\approx cT^{-1}$, where T is temperature and c is a constant) and the volume fraction of polymer, v_2 . The dotted line indicates the lower edge of the 'cap'. I, isotropic; LC, liquid crystalline.

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FIG. 2 Left, room-temperature photograph of the upper and lower phases, shown in the tube used for separating them. Right, the upper and lower phases seen between crossed polar filters.



The solvent used here was described by the supplier as 99% benzyl alcohol and was used as supplied. Gels were also made up with anhydrous benzyl alcohol (quoted as containing <0.005% water and supplied under nitrogen) and 99% benzyl alcohol that had been vacuum distilled in the laboratory and stored over a molecular sieve. No sample made with either of these latter solvents showed any sign of phase separation at any temperature. This can be attributed to the reduction of the solvent quality by water. Russo and Miller⁷ have analysed the effect of a non-solvent on the Flory phase diagram and, for simplicity, displayed the results on a binary phase diagram rather than a ternary one. They found that the phase boundary is moved up for increasing amounts of non-solvent and also that the shape of the cap is altered slightly. The trace water in our system seems to lead to a greater range of temperatures between the gel's melting point ($\sim 60^\circ\text{C}$, ref. 3) and the one-phase region, LC, and hence a larger two-phase region in which phase separation is possible once the crystals have melted. To verify this, we made up a sample of gel using anhydrous benzyl alcohol that had been deliberately contaminated with water (2% volume by volume). Again we observed phase separation, confirming that trace amounts of water shift the position of the phase diagram relative to the gel melting point and make the phase separation possible. Phase separation in the 'dry' system cannot be achieved because the melting point of the crystals (and hence the gel) is inside the single-phase region. Phase separations involving the isotropic phase (in different parts of the phase diagram) are now being investigated. □

Depletion of H_2O_2 in a Greenland ice core: implications for oxidation of volcanic SO_2

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MAJOR volcanic eruptions inject large quantities of sulphur compounds into the atmosphere¹. A principal component is sulphur dioxide; subsequent oxidation of SO_2 in the atmosphere to sulphuric acid aerosol may lead to climate perturbations². Here we present evidence to suggest that SO_2 oxidation may occur at high latitudes by reaction with H_2O_2 . Variations in sulphate and H_2O_2 concentrations in four sections of a Greenland ice core (corresponding to four volcanic events) show that high sulphate concentrations, from volcanic fallout, are accompanied by depletion of H_2O_2 . This suggests that some of the volcanic sulphur was still in the form of SO_2 when it reached Greenland, and was then oxidized by H_2O_2 in Greenland precipitation. As SO_2 oxidation in the stratosphere is very slow, volcanic events that inject SO_2 into the stratosphere could produce large depletions of distant H_2O_2 reservoirs when the material is later reinjected into the troposphere.

During volcanic eruptions sulphur compounds, predominantly H_2S and SO_2 , are released into both the troposphere and stratosphere. Once in the atmosphere, H_2S is very rapidly converted into SO_2 (ref. 3). Sulphur dioxide is then oxidized to sulphate and sulphuric acid particles by reactions occurring in the gas phase, in the aqueous phase, and/or on aerosol surfaces⁴⁻⁶. The sulphate aerosol plays a significant part in short-term climate change after volcanic eruptions, by scattering and

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1. Flory, P. J. *Proc. R. Soc. Lond.* **A234**, 73–89 (1956).
2. Miller, W. G. *et al. Pure appl. Chem.* **38**, 37–58 (1974).
3. Hill, A. & Donald, A. M. *Polymer* **29**, 1426–1432 (1988).
4. Hill, A. & Donald, A. M. *Liquid Crystals* **6**, 93–110 (1989).
5. Uematsu, I. & Uematsu, Y. *Adv. Poly. Sci.* **59**, 37–73 (1984).
6. Russo, P. S. & Miller, W. G. *Macromolecules* **16**, 1690–1693 (1983).
7. Russo, P. S. & Miller, W. G. *Macromolecules* **17**, 1324–1331 (1984).

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absorbing solar radiation⁷, which results in surface temperature cooling^{8,9}. In fact, small but sulphur-rich eruptions may produce climate changes similar to or even larger than much more voluminous silicic, sulphur-poor eruptions^{8,10,11}. As sulphur compounds are released in a reduced or incompletely oxidized form, an accurate knowledge of the production rate of sulphate aerosol from SO_2 is important for assessing the climate impact. In the aqueous phase, H_2O_2 is believed to be the most efficient oxidizing agent of SO_2 because, unlike most other species, oxidation kinetics of SO_2 by H_2O_2 are only slightly slowed down by a decreasing pH in an open system^{12,13}. Here we present a detailed record of H_2O_2 and SO_4^{2-} concentrations in four volcanic acidity layers from an ice core drilled in 1985 at Site A, central Greenland (71°N , 36°W).

Solid electroconductivity measurements were performed to locate high-conductivity layers, which presumably represent high- H^+ layers associated with volcanic events¹⁴. For chemical analyses we sampled these high-conductivity layers, as well as adjacent parts of the core corresponding to a period of 3 to 4 years before and after each eruption, at intervals of 4–7 cm (~ 2 months) using ice-cleaning procedures similar to those described by Spencer *et al.*¹⁵. Anion (Cl^- , NO_3^- , SO_4^{2-}) and cation (Na^+ , NH_4^+ , K^+ , Mg^{2+} , Ca^{2+}) concentrations were measured by ion chromatography. We determined the peroxide content of the samples using the fluorometric technique originally developed

by Guilbault *et al.*⁶ and later modified by Lazrus *et al.*¹⁷. For these samples, no distinction has been made between organic and inorganic peroxides so that all values for 'peroxide' in the samples refer to total inorganic and organic peroxides. The precision is of the order of 10% for ion chromatography measurements and 7% for peroxide measurements.

A preliminary depth-age relationship for the ice core was determined using density measurements and snow accumulation rates¹⁸. Comparisons with particle concentration measurements in a second core drilled at site A in 1985 (E. Mosley-Thompson, personal communication) confirmed that the high-acidity layers at 22.6, 36, 70.5 and 80.5 metres in the core correspond to the eruptions of Hekla (1947), Katmai (1912), Tambora (1815) and Laki (1793), as listed in Table 1. Variations in SO_4^{2-} and H_2O_2 as a function of depth for these four volcanic eruptions are shown in Fig. 1. Sulphate values have been corrected for the sea-salt contribution¹⁹ and thus represent the non-sea-salt sulphate only.

Seasonal variations in the Cl:Na ratio in polar snow have been observed¹⁹ and we used these to identify summer periods (Fig. 1). Both SO_4^{2-} and H_2O_2 exhibit seasonal variations in polar snow with peaks in the summer^{19,20}. At depth, seasonal variations in the peroxide profiles are less clear (Fig. 1) because of the diffusion of the peroxide in the ice²¹. Significant enhancements in SO_4^{2-} concentrations in the Greenland ice are observed

FIG. 1 Variation of non-sea-salt SO_4^{2-} concentration (—) and H_2O_2 concentration (----) as a function of depth for four sections of the Site A, Greenland ice core. Summer periods, based on variation of Cl:Na ratio are indicated by S.

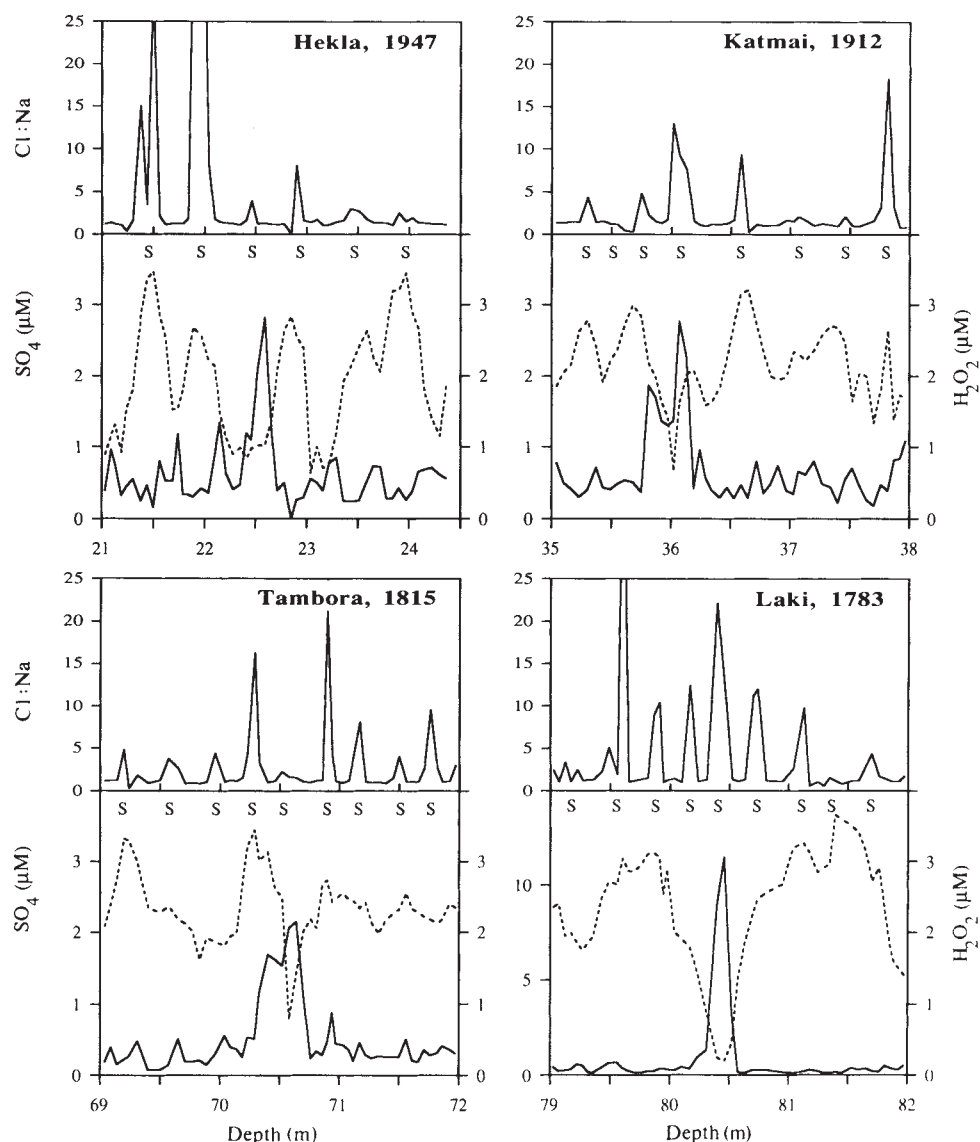


TABLE 1 Sulphur conversion rates and chemical lifetimes

Volcanic events	SO ₂ degassed* ($\times 10^{10}$ kg)	H ₂ O ₂ loss ($\mu\text{mol l}^{-1}$)	SO ₄ (volc) ($\mu\text{mol l}^{-1}$)	%SO ₂ (%)	Travel time (d)	Conversion rate ($\times 10^{-1}$ % d ⁻¹)	Lifetime (d)
Hekla 1947 Iceland	0.5	0.8	1.1	70	2–6†	0.7–2	5–15
Katmai 1912 Alaska	1–3	0.65	1.25	50	5–10‡	0.7–1.6	6–15
Tambora 1815 Indonesia	20–40	0.2	1.35	15	100–200§	0.05–0.1	100–200
Laki 1783 Iceland	15–20	2.1	6.0	35	2–6†	2–6	2–5

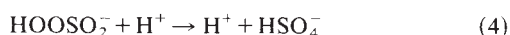
* From ref. 12.

† C. Weaver, personal communication.

‡ From ref. 27.

§ From ref. 26.

owing to the deposition of volcanic sulphuric acid, with an enrichment of up to twenty times the background for the 1783 Laki eruption. For all of the volcanic events, a distinct deviation from the expected seasonal peroxide profile is evident and very strongly correlated with increased excess SO₄²⁻ attributable to the volcanic events. This pattern is observed both for high-latitude (Laki, Hekla and Katmai) and low-latitude (Tambora) eruptions although these eruptions were chemically different^{11,22} and took place at different times of the year. The depletion of H₂O₂ associated with increased SO₄²⁻ indicates that part of the volcanic sulphur was present as SO₂ over Greenland at the time the precipitation was forming, and that a reaction occurred between H₂O₂ and SO₂ in the supercooled water film at the surface of ice crystals according to the following mechanism²³



Although we cannot rule out the possibility of H₂O₂ destruction catalysed by volcanic dust particles, all these eruptions show the same pattern despite differences in the amount of dust that reached Greenland²⁴. Furthermore, simulation of atmospheric HO₂ concentrations after the El Chichon eruption⁷ indicate that HO₂ concentration changes in the volcanic plume are insufficient to account for the H₂O₂ depletion. Examination of the Cl:Na ratio establishes that the acid deposition took place for a period of one year or more following the Tambora and Katmai events. However, H₂O₂ decreases are only observed during summer. Thus, the SO₂-H₂O₂ reaction appears to take place only during the summer. In the Laki layer, the extensive SO₂ emission may have completely depleted peroxide in the precipitation.

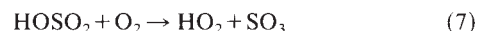
To estimate conversion rates of SO₂, we have made the assumption that all of the peroxide depletion is the result of the reaction with SO₂. According to the reaction mechanism described above, a net loss of H₂O₂ increases SO₄²⁻ concentration by the same amount. By comparing the SO₄²⁻ produced from SO₂-H₂O₂ oxidation (H₂O₂ (loss)) and the total increase in SO₄²⁻ above background (SO₄²⁻ (volc)) we can estimate the proportion of sulphur still existing as SO₂ before the SO₂-H₂O₂ reaction took place in the precipitation as

$$\% \text{SO}_2 = (\text{H}_2\text{O}_2(\text{loss}) \times 100) / \text{SO}_4^{2-}(\text{volc})$$

Thus, knowing the SO₂ proportion and assuming a reasonable travel time for SO₂ from the volcanic point source^{25,26}, we have estimated global SO₂ conversion rates for each of the events as conversion rate (% d⁻¹) = 1 - (%SO₂/100)^{1/n} where *n* is the travel time in days. Calculated values of %SO₂ and SO₂ conversion rates are presented in Table 1. The %SO₂ should be regarded as an averaged proportion of SO₂ in the volcanic cloud over Greenland during the time of deposition, which is 4–12 months, depending on the event. Chemical lifetimes can be derived from the calculated conversion rates using the following equation:

lifetime (d) = [ln(1/e)]/[ln(1 - *k*)] where *k* is the conversion rate (% d⁻¹) (Table 1).

In the case of the high-latitude eruptions, the volcanic sulphur compounds were transported to the Greenland area through the upper troposphere where the SO₂ oxidation takes place primarily in the gas phase through reaction with the OH radical by the following mechanism⁴



Over Greenland, the volcanic plume interacts with clouds resulting in the scavenging of SO₄²⁻ aerosol. Any SO₂ still present will react with H₂O₂. For these eruptions, our calculations indicate that 35–70% of the volcanic sulphur is still present as SO₂ by the time the plume reaches Greenland. The %SO₂ values are minimum estimates because there may have been insufficient H₂O₂ in the atmosphere to react with all of the volcanic SO₂, especially in the case of the Laki and the Katmai events where H₂O₂ concentration drops to almost zero. For these eruptions, calculated conversion rates are in the range 0.06–0.6% per day, corresponding to chemical lifetimes of the order of 2–15 days. These values are about ten times lower than those calculated near Mt Etna²⁷ but are very similar to those calculated for the oxidation of SO₂ in the troposphere at high latitude²⁸.

Sulphur compounds from the low-latitude Tambora eruption reach the high latitudes through stratospheric transport¹⁰. Because of the low H₂O concentration in stratospheric air, homogeneous gas-phase reactions are likely to be the main process of SO₂ oxidation²⁹. McKeen *et al.*²⁹ proposed a stratospheric oxidation mechanism similar to the previously discussed tropospheric gas-phase reaction pathway⁴. Our data indicate that 15% of the volcanic sulphur reinjected into the troposphere over the Arctic is still present as SO₂. For the Tambora event, the SO₂ oxidation rate is of the order of 0.005–0.01% per day, which is considerably lower than rates calculated for the high-latitude events. This difference between SO₂ oxidation rates in the stratosphere and the troposphere is probably due to differences in the concentrations of OH, H₂O and O₂ or N₂, which are common third molecules for reaction (6).

These low conversion rates are consistent with the SO₂ stratospheric chemical lifetime of 100–200 days observed after the 1982 El Chichon eruption³⁰ and the 1974 Vulcan de Fuego eruption³¹. The SO₂ chemical lifetime in the stratosphere during non-volcanic periods, however, is of the order of one month². Volcanic SO₂ injections therefore result in an increase in the SO₂ lifetime by a factor of 3–7. This difference between volcanic and non-volcanic periods cannot be explained by a direct decrease in the OH concentration owing to reaction (6) because the OH radical is recycled through the following reactions²⁹



But large SO₂ injections can lead to a lower O₃ photolysis by increasing the SO₂ opacity in the 300-nm wavelength region², thus indirectly affecting the OH production rate. Such a long

chemical lifetime supports the hypothesis² of a negative feedback in volcanic-induced climate perturbation, by limiting the amount of particulate H₂SO₄ present in the stratosphere at a given time.

Oxidation of volcanic SO₂ seems to be a very slow process in the stratosphere and the SO₂ chemical lifetime is increased to several months after volcanic eruptions. The volcanic SO₂ could then be oxidized far away from the eruption site and may produce a large depletion of the H₂O₂ reservoir when this material is reinjected into the troposphere.

This peroxide depletion seems to be taking place at least on

a regional scale following both high-latitude and low-latitude volcanic events. Because of the very high solubility of peroxide in water, it is very likely that this depletion is paralleled by a depletion of gas-phase peroxide. Considering that the HO₂ radical is the main source of H₂O₂ in the troposphere⁵, this may also affect the concentration or the lifetime of this reactive compound. Because of the importance of the HO₂ radical in ozone chemistry, volcanic eruptions are then likely to produce a short-term variation in both nitrogen and odd-oxygen chemistry⁷. More work will be necessary to quantify the impact on other chemical cycles in the atmosphere. □

Received 22 January; accepted 3 May 1990.

- Devine, J., Sigurdsson, H., Davis, A. & Self, S. *J. geophys. Res.* **89**, 6309–6325 (1984).
- Pinto, J. P., Turco, R. P. & Toon, O. B. *J. geophys. Res.* **94**, 11165–11174 (1989).
- Hammer, C. U., Clausen, H. B. & Dansgaard, W. *Nature* **288**, 230–235 (1980).
- Graedel, T. E. *Rev. Geophys. Space Phys.* **15**, 421–428 (1977).
- Stockwell, W. R. & Calvert, J. G. *Atmos. Environ.* **17**, 2231–2235 (1983).
- Calvert, J. G. *et al. Nature* **317**, 27–35 (1985).
- Conte, C., Devito Franco, G. & DiCastro, V. *Atmos. Environ.* **23**, 1939–1943 (1989).
- Michelangeli, D. V., Allen, M., & Yung, Y. L. *J. geophys. Res.* **94**, 18429–18443 (1989).
- Sigurdsson, H. *Proc. Conf. on Global Catastrophes in the Earth History*, Geol. Soc. of Am. Spec. Pap. 247 (1989).
- Pollack, J. B. *et al. J. geophys. Res.* **81**, 1071–1083 (1976).
- Rampino, M. R., Stothers, R. B. & Self, S. *Nature* **313**, 272–275 (1985).
- Palais, J. M. & Sigurdsson, H. *AGU Monogr.* **52** (1989).
- Kunen, S. M., Lazrus, A. L., Kok, G. J. & Heikes, B. G. *J. geophys. Res.* **88**, 3671–3674 (1983).
- Lind, J. A., Lazrus, A. L. & Kok, G. L. *J. geophys. Res.* **92**, 4171–4177 (1987).
- Spencer, M. J., Lyons, W. B., Twickler, M. S. & Mayewski, P. A. *Eos* **66**, 895–896 (1985).
- Guilbault, G. G., Birnbaum, P. & Zimmer, M. *Analyt. Chem.* **40**, 190–196 (1968).

- Lazrus, A. L., Kok, G. L., Gitlin, S. N. & Lind, J. A. *Analyt. Chem.* **57**, 917–922 (1985).
- Alley, R. B. & Koci, B. R. *Ann. Glaciol.* **10**, 30–35 (1988).
- Legrand, M. & Delmas, R. J. *J. geophys. Res.* **93**, 7153–7168 (1988).
- Sigg, A. & Neftel, A. *Ann. Glaciol.* **10**, 157–162 (1988).
- Neftel, A., Jacob, P. & Klockow, D. *Tellus* **B38**, 262–270 (1986).
- Herron, M. M. *J. geophys. Res.* **87**, 3052–3060 (1982).
- Hoffmann, M. R. & Edwards, J. O. *J. phys. Chem.* **79**, 2096–2098 (1975).
- Sigurdsson, H., Devine, J. & Davis, A. *Jokull* **35**, 1–8 (1985).
- McKormick, M. P. & Swissler, T. J. *Geophys. Res. Lett.* **10**, 877–880 (1983).
- Bridgman, H. A., Schnell, R. C., Khal, J. D., Herbert, G. A. & Joranger, E. *Atmos. Environ.* **23**, 2537–2549 (1989).
- Martin, D. *et al. J. geophys. Res.* **91**, 12249–12254 (1986).
- Altshuler, A. P. *Atmos. Environ.* **13**, 1653–1661 (1983).
- McKeen, S. A., Liu, S. C. & Kiang, C. S. *J. geophys. Res.* **89**, 4873–4881 (1984).
- Krueger, A. J. *Science* **220**, 1377–1379 (1983).
- Lazrus, A. L. *et al. J. geophys. Res.* **84**, 7869–7875 (1979).

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The sensitivity of terrestrial carbon storage to climate change

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THE high correlation found between atmospheric CO₂ concentration and temperature during the past 160,000 years^{1,2} implicates CO₂ as an important driving force behind changes in palaeoclimate. Changes in biological processes and circulation patterns in the oceans are believed to drive glacial-to-interglacial changes in CO₂ and thus in climate. Here we estimate the role of the terrestrial biosphere in controlling atmospheric CO₂ levels during climate perturbations. By considering the coupling between vegetation distributions and climate, we perform simulations to calculate the global geographical distribution of vegetation during the last glacial maximum, 18 kyr ago. The known changes in sea level at this time, together with the simulated climate-driven spatial arrangement of vegetation, result in a mass transfer of carbon from the terrestrial biosphere to the atmosphere ranging from 30 × 10⁹ tonnes (30 Gt, corresponding to 15 p.p.m. CO₂) to –50 Gt (25 p.p.m.). Thus, although the biosphere may have contributed to the decrease in atmospheric CO₂ of 80 p.p.m. known to have occurred at 18 kyr (refs 1, 2), it does not seem to have been a dominant factor. For simulations run with twice the present-day CO₂ levels, strong negative feedbacks appear which remove 235 Gt of carbon (128 p.p.m. CO₂) from the atmosphere.

Bioclimate schemes relate the distribution of climates and the distribution of vegetation types, classifying climates according to their thermal and moisture attributes. The climate classes are named for vegetation types with which they have been associated. This association of vegetation and climate permits the simulation of vegetation distributions for different climates.

In an earlier study³, the contemporary distribution of global vegetation was simulated by using a refined version of the

Holdridge Life Zone Scheme⁴ to classify observed 30-year mean annual temperature and precipitation⁵. The climate classes were grouped and named for the vegetation types with which they have greatest geographic coincidence⁶. Unlike earlier bioclimate predictions^{7–10}, resulting climate classes were named for present-day vegetation types defined by the internationally recognized UNESCO classification scheme¹¹ rather than for the independent and generalized vegetation groups used by the bioclimate schemes. Quantitative evaluation of the simulated distribution is thus possible because a consistent vegetation terminology is used for both observed and simulated vegetation. At 1° × 1° resolution for the globe, 77% of the simulated landscape, classified as 29 vegetation types, spatially corresponded with observed vegetation. The misclassifications over 23% of the land surface generally involve climatically similar vegetation types which also have similar carbon attributes.

For the study reported here, the 29 vegetation types are combined into 14 types to reflect more generalized units of carbon storage. We apply distribution relationships developed between the 14 observed UNESCO types and the observed climatology to perturbed climates. As well as today's climates, climates at 18 kyr BP (before present) and climates for doubled CO₂ levels (2 × CO₂) have been simulated using the general circulation model (GCM) at the Goddard Institute for Space Studies¹². Temperature and precipitation, simulated at a 4° × 5° resolution, are linearly interpolated to 2½°. Both the 2½° observed and simulated distributions are then regridded to 1° without further interpolation beyond that necessary for the ½° registration. Annual temperature and precipitation used here are calculated for each 1° gridbox by adding the difference between simulated perturbed and simulated present climates to observed 30-year mean climatologies⁵. In this way, the interpretation of bioclimate patterns is rooted in observed climatology. Land area changes as 1°-gridded surface areas emerge or submerge with altered sea level. The sea level at 18 kyr is lowered by 130 and 100 m to bracket a best estimate of 121 m (ref. 13), and the sea level for the 2 × CO₂ simulation is raised by 2 m, a likely upper estimate¹⁴.

To evaluate the possible impact of the perturbed biosphere on the carbon cycle, we calculate the sizes of the carbon reser-

TABLE 1 Carbon densities and areal extents for 14 vegetation types

Vegetation type	Biomass (kg C m ⁻²)		Soil carbon (kg C m ⁻²)		Present area (10 ¹² m ²)	18 kyr BP (-130 m) (% change)	18 kyr BP (-100 m) (% change)	2 × CO ₂ (+2 m) (% change)
	ref. 16	ref. 15	ref. 19*	refs 17, 18				
1	15	18	10	10	19	20	17	75
2	5	5	7	8	6	-23	-31	-42
3	3	2	6	12	5	-40	-42	35
4	1	0	6	7	30	2	0	-22
5	0	0	3	1	14	-91	-91	-62
6	3	1	11	20	2	-7	-8	-17
7	3	3	8	29	2	78	76	4
8	10	11	10	10	12	32	31	40
9	9	9	14	18	16	-16	-18	5
10	6	6	12	18	6	-21	-22	-66
11	1	0	17	13	7	2	1	-63
12	0	0	0	0	3	1,223	1,171	0
13	8	13	8	11	2	170	163	-31
14	6	11	7	8	9	-89	-91	21
Global (Gt C)	748	834	1,143	1,313	132	152 × 10 ¹² m ²	148 × 10 ¹² m ²	132 × 10 ¹² m ²

The 14 simulated types are listed in column 1. They are: (1) tropical rain forest, (2) drought-deciduous woodland, (3) savanna, (4) arid grassland and shrubland, (5) desert, (6) mesic grassland, (7) Mediterranean forest and woodland, (8) cold-deciduous broad-leaved forest and woodland, (9) cold-deciduous needle-leaved forest and woodland, (10) evergreen needle-leaved forest and woodland, (11) tundra, (12) polar desert and ice, (13) temperate evergreen seasonal broad-leaved forest, (14) drought-deciduous and drought-seasonal broad-leaved forest. Carbon densities (columns 2–5) are based on published figures. They are constant for all climates. Areal extents of the 14 vegetation types (column 6) were bioclimatically simulated using observed climatology⁴. Simulated changes in areal extents (columns 7–9) are expressed as a percentage of present-day area (excluding Antarctica).

* Soil carbon is estimated directly from climate and averaged over the area of each vegetation type simulated from observed climate.

voirs in vegetation and soils at 18 kyr, for 2 × CO₂ and for the present climate. Biomass estimates for vegetation types are taken from Whittaker and Likens¹⁵ as well as from Olson *et al.*¹⁶. Soil carbon densities for their associated soils types are obtained by combining estimates of Ajtay *et al.*¹⁷ and Schlesinger¹⁸. These carbon densities are geographic extrapolations of field measurements. The distribution of soil carbon densities is also estimated directly from climate using Post *et al.*'s superposition of isolines of soil carbon densities on the Holdridge climate space¹⁹.

Over 75% and 60% of the present vegetated landscape was altered by the 18 kyr BP and 2 × CO₂ simulated climates respectively. During the 18 kyr BP simulation, land temperatures dropped ~4.4 °C in the Southern Hemisphere and 8.5 °C in the Northern Hemisphere. Although the global landscape was more arid, eastern and southern coasts as well as present arid regions were wetter than today. The 100- and 130-m drop in sea level increased the land area by 12% and 15%. Regions of extreme climates (deserts, tropical rainforests and ice) show greatest sensitivity to climate change.

In this study, the biospheric carbon changes only because the distribution of vegetation types changes. The dynamics involved in both the change in the distribution of vegetation and changes in carbon density in response to changing climate are not considered. This analysis is a comparison of static snapshots of potential vegetation existing for today's, for the 18 kyr BP and for a 2 × CO₂, climate.

Table 1 lists carbon densities for the 14 vegetation types (columns 2–5) as well as their present land areas (column 6) and percentage change in their areas associated with perturbed climates. (Numbers in the discussion refer to Olson *et al.*¹⁶ and Post *et al.*¹⁹ unless otherwise stated.) During the 18 kyr BP (-130 m) simulation, the advance of ice sheets claimed 25 × 10¹² m² of present land, significantly reducing the extent of boreal vegetation. The areas of needle-leaved boreal forests (types 9 and 10) were reduced by 17% despite a 5% expansion on newly emerged land. The descent of the ice sheets and other effects of unfavourable climate as well as sea-level changes reduce the carbon stored in the 18 kyr BP boreal forests (type 9 and 10) by 80 Gt (30 Gt as biomass carbon and 50 Gt as soil carbon).

During the 18 kyr BP simulation, a large conversion of carbon

occurred in the present land area of the equatorial region and in the Southern Hemisphere. Temperate and tropical broad-leaved forests (types 7, 8, 1, and 13) expanded into the cooler and wetter subtropical regions displacing subtropical broad-leaved forests and savannas (types 2, 14 and 3) which exist in climates having more strongly seasonal precipitation. The areal expansion of the less drought-seasonal forests (types 7, 8, 1 and 13) on existing and new land increases their carbon storage by 256 Gt, which is about equally divided between biomass and soil and also equally divided between new and existing land. The areas of the more drought-seasonal vegetation (types 2, 14 and 6) were reduced by 57% shrinking their carbon pool by 136 Gt (57 Gt carbon in biomass and 79 Gt as soil carbon).

Because of increased precipitation in arid regions during the 18 kyr BP simulations the area of deserts is reduced by 91%. Although 18 kyr BP desert decreases by 12 × 10¹² m², this large area reduction represents only a 45 Gt decrease in the desert carbon pool—almost entirely as soil carbon.

Although the simulated biosphere responds dramatically to 18 kyr BP changes in climate, large regional changes in carbon reservoirs and changes in soil and biomass carbon offset one another in the global carbon budget. Global estimates of changes in carbon reservoirs from present to perturbed climates are listed in Table 2. Estimates of carbon-reservoir changes in Table 2 are broken into those effected by changes in both climate and sea level and those associated with changes in climate only. For 18 kyr BP climate with a 130 m drop in sea level, the 6% (43 Gt) gain in biomass carbon is offset by the 4% (43 Gt) decline in the soil carbon pool. Net change for the amount of carbon held in the 18 kyr BP (-130 m) biosphere is approximately zero. Like the changes in the biomass and soil reservoir, the changes in the global carbon reservoir associated with climate are also compensated by the effects of sea-level change on the biosphere. The direct effects of unfavourable 18 kyr BP climate reduce biomass carbon by 7% and soil carbon by 13%. This 200-Gt reduction of terrestrial biospheric carbon is offset by the development of vegetation and soil on new land. The 20 × 10¹² m² of newly emerged land (Table 1) increases the carbon in the present terrestrial biomass by 13% and soil carbon by 9%. The amount and location of emerged land can account for a large increase in biospheric carbon. In this experiment, the biospheric

TABLE 2 Global terrestrial carbon reservoir changes (%) relative to today

Climate	Reservoir	Present-day carbon reservoir (Gt C)	Percent change in carbon reservoir			
			change in sea level (m)			
			-130	-100	0*	+2
18 kyr BP	biomass					
	ref. 16	748	6	3	-7	—
	ref. 15	834	5	2	-8	—
	soil carbon					
2×CO ₂	ref. 19	1,143	-4	-6	-13	—
	refs 17, 18	1,313	-1	-3	-10	—
	biomass					
	ref. 16	748	—	—	31	31
	ref. 15	834	—	—	35	35
	soil carbon					
	ref. 19	1,143	—	—	1	1
	refs 17, 18	1,313	—	—	3	3

* Reservoir changes brought about by direct climate effects only

carbon supported on new land is as great as the changes in the biospheric carbon resulting directly from climate.

Similar changes in biospheric carbon reservoirs occurred in the 18 kyr BP (-100 m) simulation but the changes were affected by the smaller increase in land area associated with the 100 m drop in sea level. Biomass carbon storage increases by 3% (25 Gt) relative to the present reservoir whereas soils lose 6% (65 Gt). The terrestrial biosphere in this experiment loses 40 Gt of carbon.

The land climate for the 2×CO₂ simulation is an average of 5.4 °C warmer and is 17.5 mm d⁻¹ wetter than present climate. Areas of tropical rainforests increase by 75% (Table 1) increasing their biomass and soil carbon reservoirs by 215 Gt and 130 Gt of carbon respectively. Desert (type 5) and semi-desert (type 4) regions are reduced by 60% and 20%, reducing the carbon stored in arid lands by 75 Gt. Cold-deciduous broad-leaved (type 8) forests expand northward in the Northern Hemisphere in the warmer, wetter 2×CO₂ climate adding 50 Gt carbon to their soil reservoirs and 60 Gt to their biomass. Areal decrease of boreal evergreen needleleaved forests (type 10) and tundra (type 11) reduces their soil carbon pool by 145 Gt.

The global terrestrial biosphere responds to the 2×CO₂ climate with a 31% increase in biomass a 1% increase in soil carbon. This 235-Gt carbon expansion of the terrestrial biosphere is due entirely to the change in climate (the sea level rises 2 m).

Although the spatial rearrangement of vegetation in response to perturbed climates is significant, the magnitude and direction of the net change in carbon storage remain unclear. Many caveats must accompany the resulting global carbon budgets. (1) At present, only 77% of the observed vegetated landscape can be replicated because we do not know how to express climate to define best the structural/functional attributes of vegetation. In addition, the secondary effects of soils, topography, humans and other environmental factors on the vegetation distribution are not accounted for in this study. Because misclassification of vegetation most often occurs between climatically similar vegetation types having similar carbon-storage attributes, the study's results are not highly sensitive to small errors in classification. Results are most sensitive to misclassifications in areas having a strong gradient in carbon density (for example, along a north-south transect of soil through ice, tundra and boreal forests). (2) We assume relationships now existing between climate classes and vegetation types hold for perturbed climates. In fact vegetation types existing under perturbed climates may differ in structure and function from those existing today. We also assume that vegetation is in equilibrium with climate — a condition which is never entirely met in a constantly changing climate. The assumption of steady state becomes less correct for the more slowly changing soil-carbon reservoir over

what is believed to be the relatively short time period of an approaching 2×CO₂ climate. (3) The estimation and extrapolation of carbon densities for the geographically extensive and diverse vegetation types requires more field measurements than are presently available. Sensitivity of global terrestrial carbon storage to small errors in the carbon-storage description (Table 1) increases with the area of the vegetation type. Such errors are likely to increase with the density of carbon storage, with increasing percentage of storage in the soil (where carbon density is more difficult to measure), with diversity of carbon storage within vegetation type (making extrapolation of field measurements difficult) and inversely with the frequency of the field sampling of carbon density. The discrepancies between the minimum terrestrial carbon storage^{16,19} shown in Table 2 and the maximum values^{15,17,18} for a single-climate scenario are generally more than double the storage differences between climate scenarios. (4) Uncertainties and errors in GCM simulations, especially of precipitation affect the distribution of vegetation and terrestrial carbon. (5) At the GCM level of generalization and structural homogenization of vegetation types, issues of CO₂ fertilization and the functional differences of C₃ and C₄ plants cannot be addressed. (6) The 100-m and 130-m drops in sea level in the 18 kyr BP simulations are responsible for a 40-Gt difference in the amount of carbon stored in the biosphere. (7) Estimates of the changes in the net global biospheric carbon are obtained by summing large numbers often having opposite signs. Because there is a large margin of error in each component number, an accurate net change in the global terrestrial biosphere is difficult to obtain. Some generalizations, however, are possible.

In our study, both biomass and soil carbon pools during the 18 kyr BP (-130 m) simulation decreased by 200 Gt carbon on the present land area, or ~10% of the total present land reservoir. The decrease is countered by the substantial increase of carbon stored on the newly emerged land. The magnitude of this compensation is difficult to ascertain because of the uncertainties in the sea-level change and lack of understanding of the biosphere on the new landscape. By assuming a sea-level drop of 130 m and applying today's terrestrial carbon relationships, we obtain an increase in carbon storage of 200 Gt on new land, exactly compensating for the loss owing to unfavourable climate. Although our best estimate of the mass transfer of carbon between the biosphere and the atmosphere for the 18 kyr BP simulation is approximately zero, Tables 1 and 2 show possible biospheric changes ranging from an increase of 30 Gt carbon for -130 m (refs 16-18) to a decrease of 50 Gt carbon for the -100-m experiment^{15,19}. Although biospheric changes do not dominate the 82-p.p.m. (165 Gt carbon) difference in atmospheric CO₂ between the Holocene and 18 kyr BP found in ice cores, their contribution may not be negligible because of uncertainties in calculating the components of large compensating factors in the biosphere-atmosphere carbon flux.

In our 2×CO₂ simulation, the change in sea level (+2 m) has a negligible effect on global land area. In this experiment, a terrestrial biosphere in equilibrium with a 2×CO₂ climate has an increased carbon storage ranging from 338 Gt (refs 15, 17, 18) to 245 Gt (refs 16, 19), or 169 p.p.m. to 122 p.p.m. This represents a potentially strong negative feedback on the atmospheric CO₂ increase.

In both cold and warm climates, the redistribution of vegetation results in carbon transfers from soil to biomass and from boreal to tropical regions. The present climate seems to be unique in its capacity to support such extensive carbon-rich boreal soils and such extensive carbon-poor arid lands.

These sensitivity experiments explore the influence the terrestrial biosphere might have had in the glacial-interglacial changes of atmospheric CO₂ as well as the possible carbon-storage role in a 2×CO₂ climate. We hope that this research can provide a framework to analyse and extrapolate terrestrial response to global climate changes. □

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1. Barnola, J. M., Raynaud, D., Korotkevich, Y. S. & Lorius, C. *Nature* **329**, 408–414 (1987).
2. Jouzel, J. *et al.* *Nature* **329**, 403–408 (1987).
3. Prentice, K. C. *J. geophys. Res.* (in the press).
4. Holdridge, L. R. *Science* **105**, 367–368 (1947).
5. Shea, D. J. *NCAR Technical Note* 269 + STR (Natn. Centre atmos. Res., Boulder, 1986).
6. Matthews, E. *J. Clim. appl. Met.* **22**, 474–487 (1983).
7. Manabe, S. & Stouffer, R. J. *J. geophys. Res.* **85**, 5529–5554 (1980).
8. Hansen, J. *et al.* *Geophys. Monogr. Ser. (M. Ewing Symp. 5)* **29**, 130–163 (1984).
9. Lashof, D. thesis Univ. California, Berkeley (1987).
10. Emanuel, W. R., Shugart, H. H. & Stevenson, M. P. *Climatic Change* **7**, 29–43 (1985).
11. UNESCO, *International Classification and Mapping of Vegetation* (UNESCO, Paris, 1973).
12. Hansen, J. *et al.* *Mon. Wea. Rev.* **111**, 609–662 (1983).
13. Fairbanks, R. G. *Nature* **342**, 637–642 (1989).
14. Oerlemans, J. *Clim. Change* **15**, 151–174 (1989).
15. Whittaker, R. H. & Likens, G. E. in *Primary Productivity of the Biosphere* (eds Lieth, H. & Whittaker, R. H.) 305–328 (Springer-Verlag, New York, 1975).
16. Olson, J. S., Watts, J. A. & Allison, L. J. *US Dept. of Energy DOE/NRB-037* (1983).
17. Aitay, G. L., Ketner, P. & Duvigneaud, P. *SCOPE 13* (eds Bolin, B., Degens, E. T., Kemps, S. & Ketner, P.) 129–181 (Wiley, New York, 1979).
18. Schlesinger, W. in *The Role of Terrestrial Vegetation in the Global Carbon Cycle* (ed. Woodwell, G. M.) (Wiley, New York, 1979).
19. Post, W. M., Emanuel, W. R., Zinke, P. J. & Stragenberger, A. G. *Nature* **298**, 156–159 (1982).

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Temperature measurements during initiation and growth of a black smoker chimney

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BLACK smoker chimneys are hollow spires formed by mineral deposition at sea-floor hydrothermal vent sites. They grow in two stages: formation of a sulphate-dominated wall (stage I), followed by precipitation of sulphide minerals on the inner side, and in pore spaces, of the wall (stage II)^{1,2}. Here we present the results of an *in situ* 46-day experiment which allows direct observation of stage I growth processes by monitoring wall and fluid temperatures. The position and thickness of stage I chimney walls can change on short timescales, and are controlled by the dynamics of fluid flow both during³ and after initial wall emplacement. The temperature in the main flow remained stable at $353 \pm 2^\circ\text{C}$ throughout the experiment, including the period during which the chimney wall was constructed. Six thermocouples, however, recorded maximum temperatures between 365 and 405°C , which are significantly higher than most exit temperatures documented previously⁴. These latter observations bear on the question of the maximum temperatures attainable in sea-floor hydrothermal systems (refs 4–9, J. R. Delaney, manuscript in preparation).

In July 1988 an instrument equipped with an array of thermocouples housed within a test collar was deployed in the Endeavour hydrothermal vent field ($47^\circ 57' \text{N}$) on the Juan de Fuca Ridge^{10,11}. The collar (Fig. 1a) was set over the opening of a black smoker vent and remained in place for 46 days. Temperatures (Fig. 2) were recorded every 2 min by 10 thermocouples inside the collar, 2 thermocouples outside the collar, and a platinum resistance temperature device (RTD) on the instrument platform, which was moored 1 m from the collar. The presence of the collar and thermocouples probably influenced fluid flow and mineral deposition to some extent, but some disturbance is unavoidable.

During the first 52 hours of the experiment, before chimney growth, the thermal structure of the hydrothermal plume was documented. Temperatures at TC02 (thermocouple 2), TC03 and TC09 increased rapidly to, and remained stable at, $353 \pm 2^\circ\text{C}$. Temperatures increased more slowly to maxima of $353 \pm$

TABLE 1 Pressure difference across chimney wall

Diameter (m)	Volume flow rate ($\text{m}^3 \text{s}^{-1}$)	Pressure difference (Pa)
0.016	0.00039	0
	0.00069	6,700
0.02	0.00039	–2,200
	0.00069	0
0.025	0.00039	–2,900
	0.00069	–2,200

Pressure differences calculated using equation (2), using $y=1 \text{ m}$ and $f=0.04$ (ref. 3); densities for 2°C sea water and 350°C hydrothermal fluid are $1,003$ and 674 kg m^{-3} (ref. 12), respectively.

2°C at TC05, TC06 and TC11, 365°C at TC08, 375°C at TC10, 381°C at TC07, and 405°C at TC04. The three highest temperatures are on the boiling curve (375°C) or in the two-phase region for a seawater-salinity fluid at 220 bars^{6,12}. Excursions to $\geq 375^\circ\text{C}$ lasted for 90 min at TC04, 42 min at TC07 and 4 min at TC10, and were generally coincident but not simultaneous. In 1984 temperatures of 370 – $>400^\circ\text{C}$ and of $>400^\circ\text{C}$ were measured at this site (Endeavour hydrothermal field; refs 6, 7, J. R. Delaney, manuscript in preparation) but their reliability has been disputed because these values were not reproduced on subsequent visits (temperatures were lower, but the same individual vents were not occupied) (ref. 4, J. R. Delaney, manuscript in preparation). In 1988 a temperature of 375°C was measured 2 km to the north (ref. 5, J. R. Delaney, manuscript in preparation). Nearly all other published exit temperatures are $<350 \pm 5^\circ\text{C}$ (ref. 4), including the temperature of the main flow measured in this experiment. Experimental reproduction of hydrothermal fluid compositions, however, has only been accomplished at temperatures $>385^\circ\text{C}$ (refs 8, 9). Our high-temperature measurements seem to be reliable because they were all made adjacent to one another (Fig. 1a), and all thermocouples recorded temperatures within 2 – 4°C of one another (within the error limits, $\pm 2^\circ\text{C}$) during deployment and recovery, and seem to have performed consistently throughout all other portions of the experiment. On the other hand, thermocouple TC02, only 4 cm from TC04, recorded a stable temperature of $353 \pm 2^\circ\text{C}$ for the duration of the experiment, including the period during which TC04 recorded 405°C , and the thermocouples that measured anomalously high values did not record stable temperatures or temperatures similar to one another (Fig. 1b). The simplest explanation that accounts for all measurements is that there were two sources, one that was very stable at 353°C , and a second, hotter source which was responsible for the $>355^\circ\text{C}$ temperatures (J. R. Delaney, manuscript in preparation).

The anomalously high temperatures were recorded only during a short time following emplacement of the collar. Within 7 hours, temperatures at TC04, TC07, TC08 and TC10 became relatively stable at 369, 361, 358 and 357°C respectively, and drifted to 363, 356, 354 and 354°C over the next 45 hours. During this time all other thermocouples in the collar measured stable temperatures of $353 \pm 2^\circ\text{C}$ (Fig. 1c, Fig. 2a). The initial rapid increases to $353 \pm 2^\circ\text{C}$ at TC02, TC03 and TC09 indicate that hot fluid was directed against one side of the test collar (Fig. 1). Progressive increases in temperatures at the other thermocouples to $>350^\circ\text{C}$ probably reflect decreases in leakage of sea water into the collar as mineral precipitation at the collar base sealed openings.

During the third day of the experiment, temperatures at all thermocouples except TC02 and TC03 began to change rapidly (Fig. 2b), indicating the sudden onset of seawater entrainment over the top of the collar (Fig. 1d). The cause of entrainment is unclear but possibilities include development of an instability in the turbulent plume, or a sudden decrease in flow rate. The

data show that once sea water was entrained, a barrier formed rapidly (in 6–12 h; Fig. 2b), isolating TC06, TC07 and TC11 from the fluid. Over the following 3.5 days, temperatures at TC06, TC07, TC10 and TC11 decreased to $<106^{\circ}\text{C}$, and temperatures at TC05, TC04, TC09 and TC08 fluctuated rapidly, consistent with being in zones of mixing. TC03 also showed minor fluctuations, at one point recording a temperature of 372°C ; TC05 recorded 372°C 30 min before the TC03 measurement. Throughout this time, and during wall emplacement, TC02 remained stable at $353 \pm 2^{\circ}\text{C}$. After 3.5 days, temperatures at each thermocouple became relatively constant. TC08 recorded a temperature of 309°C , consistent with being located in the newly formed chimney wall; TC09 and TC04 recorded temperatures of 342 and 343°C , indicating that they were in or

against the inner side of the chimney wall, and TC05 and TC03 recorded stable temperatures of $353 \pm 2^{\circ}\text{C}$, indicative of being in rapidly moving high-temperature fluid.

During the remaining 40 days of the experiment temperatures at TC06, TC07, TC10 and TC11 decreased to ambient temperatures (Fig. 2a) as the wall became impermeable to leakage of hydrothermal fluid. Temperatures at TC02 and TC03 remained stable at $353 \pm 2^{\circ}\text{C}$, and temperatures at TC04, TC08, TC05 and TC09 decreased to 21 , 67 , 134 and 283°C , respectively. On recovery, TC04 was outside the wall, TC08 was against the outer wall, and TC05 and TC09 were embedded in the wall (Fig. 1h). The final locations of these thermocouples and the recorded decreases in temperature with time are most easily accounted for by inward thickening of the wall coupled with

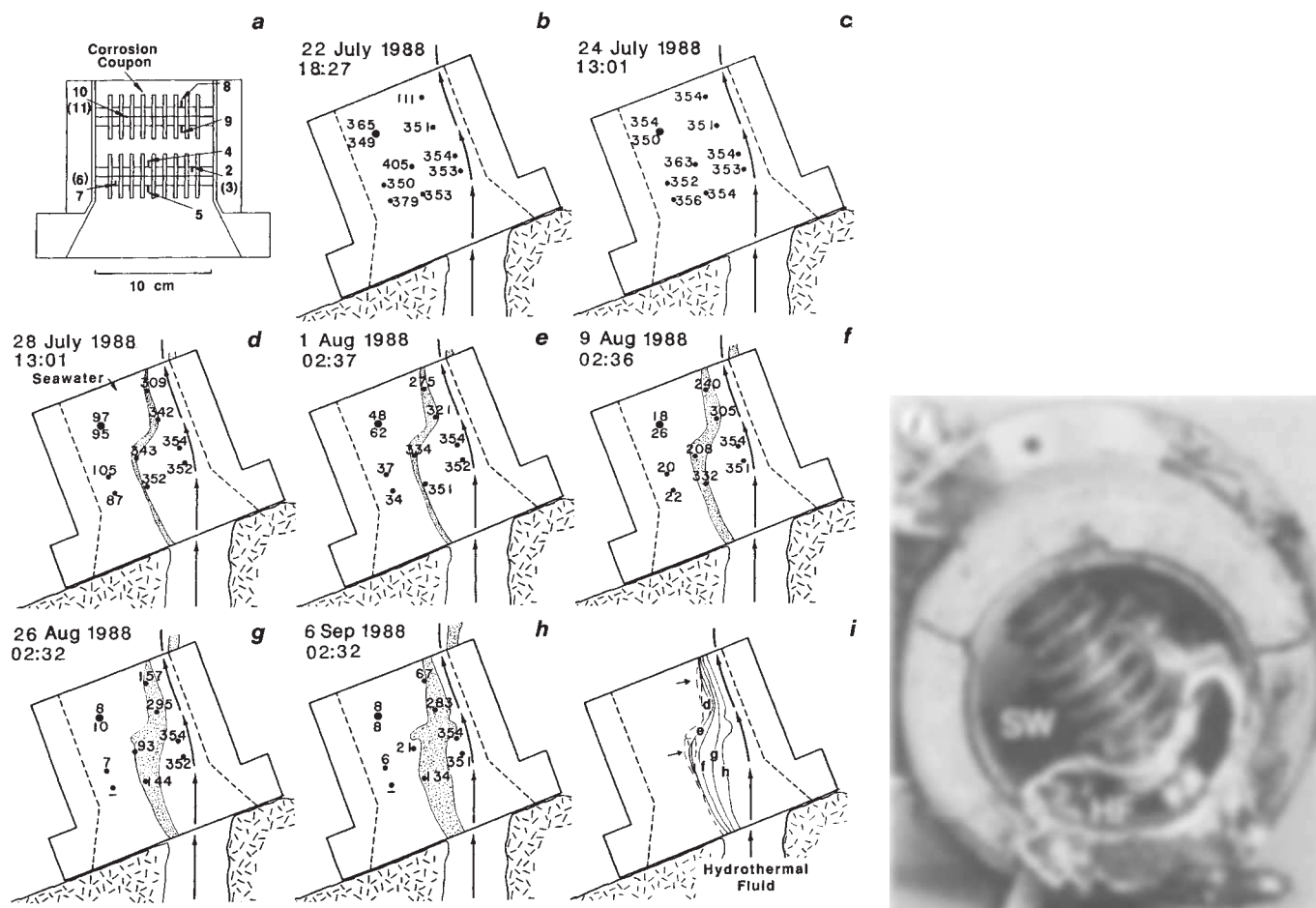


FIG. 1 a, Cross-sectional view of the test collar, shown parallel to thermocouple-coupon arrays. Numbers refer to thermocouple locations within the collar; parentheses indicate location on opposite side. The collar consisted of a 15.3-cm-high, 10-cm-inner-diameter stainless steel cylinder insulated with a 2.54-cm outer ring of mortar. It housed the temperature-sensing ends of 13 thermocouples and 3 sets of metal coupons, used to study the corrosive effects of hydrothermal fluid on construction materials²². A 6-m-long umbilical cable, which contained the thermocouple leads, linked the test collar to a digital sampling and storage system consisting of a microprocessor, solid-state memory and battery pack. Corrosion coupons exposed to fluids included nickel and titanium alloys, and stainless steel. Only stainless steel coupons exhibited corrosion. b–g, Cross-sectional view of collar showing measured temperatures and inferred wall positions (stippled) during the experiment. The 20° angle of the test collar from vertical represents the inferred orientation during the experiment, based on observations of flow during deployment, and on the 20° angle of the uppermost chimney wall to the test collar on recovery. h, Temperatures recorded before instrument recovery, and location of the wall inside the collar on recovery. i, Location of the inner (solid lines) and outer (dashed lines) chimney wall as a function of time; wall positions are taken from d–h, and are labelled as such. Arrows indicate places on outer wall subject to dissolution. Dashed

lines are, from left to right, from d, g and h. j, Chimney wall constructed in the test collar, viewed from above. Upper portions of the wall collapsed during instrument recovery. The chimney lapped onto the stainless steel collar wall, partitioning the cross-sectional area into two portions, one subject to high-temperature flow (HF), and the other dominated by cold sea water (SW). Composition was determined using transmitted- and reflected-light microscopy and X-ray diffraction analysis. The wall ranges in thickness from <1 to 30 mm and is mineralogically and texturally similar to the uppermost outer layers of more mature chimneys^{1,2,23–25}. The dominant mineral present is anhydrite, ranging in size from $<10\text{ }\mu\text{m}$ to $>1\text{ mm}$, averaging $500\text{ }\mu\text{m}$. Fine grains ($<5\text{--}20\text{ }\mu\text{m}$) of pyrrhotite, pyrite, chalcocopyrite and sphalerite or wurtzite intergrown with and included in anhydrite are present in small amounts ($<5\text{--}10\%$). Minerals do not exhibit any signs of replacement. A thin ($<1\text{ mm}$) partially continuous layer of chalcocopyrite is present on the inner side of the wall. At some locations, ridges of anhydrite project into the open channel. X-ray diffraction analyses of the thickest portion of the wall indicate the presence of caminite (magnesium hydroxysulphate hydrate). The only other reported occurrence of caminite, which was also intergrown with anhydrite, was in the outer portion of a black smoker chimney wall from 21°N on the East Pacific Rise^{26–28}.

some dissolution and removal of the outer wall at TC04 and TC08. Recorded temperatures and inferred wall positions for six different times are displayed in Fig. 1.

The experiment allowed the first direct observation of a very young chimney wall. The wall that grew up through the collar is composed dominantly of anhydrite (Fig. 1j). Only a very thin (<1 mm) layer of chalcopyrite is present against the inner side of the wall, and the few sulphide grains intergrown with and included in anhydrite do not exhibit any signs of replacement. The time-series records indicate that the wall was emplaced on a timescale of days, consistent with previous observations^{2,13}, and that it then thickened inward by as much as 2.5 cm over a time interval of weeks (Fig. 1i). The lack of replacement of earlier formed minerals, and presence of only minor amounts of chalcopyrite lining the innermost (youngest) chimney wall, indicate that stage I growth prevailed. Inward growth was dominated by anhydrite deposition, which requires the presence of sulphate, a component available in sea water but not in hydrothermal fluid¹⁴. If sulphate is supplied by the inward advection of sea water, then the rate of thickening is controlled by the magnitude of the pressure gradient across the wall, and by permeability K as expressed by Darcy's law

$$u = -\frac{K P_i - P_o}{\eta \, dx} \quad (1)$$

where u is advection rate, η is viscosity, dx is the width of the porous medium, and P_i and P_o are pressure on the inner and outer sides of the wall¹⁵. Inward growth by deposition of anhydrite should cease when P_i becomes equal to or greater than P_o .

The magnitude of the pressure difference required to produce the observed thickening of the wall can be estimated by considering (1) the flux of sulphate necessary to account for deposition of a 0.025-m-thick wall of anhydrite (molar volume $4.6 \times 10^{-5} \text{ m}^3 \text{ mol}^{-1}$; ref. 16) in 40 days, which is $1.6 \times 10^{-4} \text{ mol m}^{-2} \text{ s}^{-1}$, and (2) the advection rate of sulphate (concentration in sea water of 28 mol m^{-3} ; ref. 17) necessary to provide this flux, $5.7 \times 10^{-6} \text{ m s}^{-1}$. This advection rate could be generated by a pressure difference of $-2,253 \text{ Pa}$ across a 0.025-m-thick wall (calculated using equation (1)), assuming an average viscosity of hot seawater-salinity fluid of $5 \times 10^{-4} \text{ Pa s}$ (ref. 18), and a permeability similar to that of sandstone ($10^{-13.5} \text{ m}^2$; ref. 14). Conditions under which such a pressure difference

would exist can be evaluated using a method that considers the fluid dynamics of high-velocity flow inside the chimney channel³. The pressure difference inside a chimney of constant diameter d can be calculated by summing the buoyancy force with a friction loss term

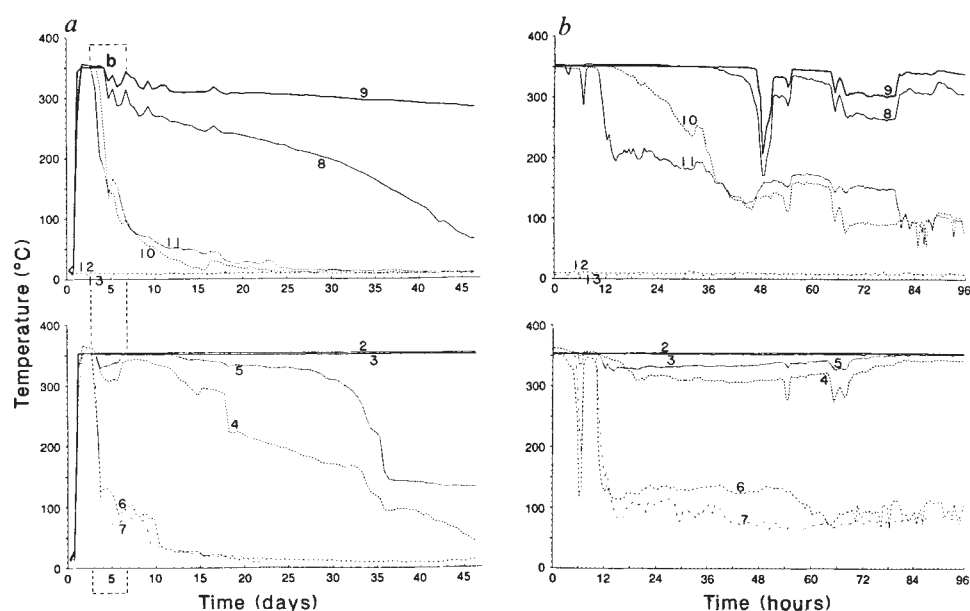
$$P_i - P_o = (\rho_i - \rho_o)gy + \frac{8f\rho_i Q^2}{\pi^2 d^5} y \quad (2)$$

where ρ is fluid density, y is depth in the channel, f is a friction factor, and Q is volume flow rate³. Calculated pressure differences at a depth of 1 m from the top of the chimney as functions of Q and d are given in Table 1. The value of d that corresponds to a pressure difference of zero for a given constant flow rate is termed the 'stable' chimney diameter³. A pressure difference of the order of $-2,200 \text{ Pa}$ at a depth of 1 m in the channel, which would permit inward thickening, could result either from a decrease in volume flow rate after initial emplacement at a stable diameter³, or from emplacement of the wall at a diameter larger than this. The larger diameter could occur if wall emplacement mimics the outer bound of the plume which expands above the chimney orifice¹⁹. In either case inward growth would cease when a stable diameter for the existing volume flow rate Q is achieved.

The idea of a stable diameter can also be used to explain why the chimney wall grew within the test collar instead of on top of it. Formation of a chimney wall with a stable diameter of 10 cm would require an extremely high flow rate of 26 kg s^{-1} ($0.0384 \text{ m}^3 \text{ s}^{-1}$). Estimated mass flow rates from vigorous smokers at 21° N on the East Pacific Rise are significantly less, of the order of 1.5 kg s^{-1} (ref. 19). The wall formed within the test collar had a hydraulic radius (cross-sectional area/perimeter) of 0.005 m, equivalent to a circular diameter of 0.02 m, which corresponds to a more reasonable flow rate of 0.5 kg s^{-1} ($0.00069 \text{ m}^3 \text{ s}^{-1}$). The experiment indicates that the orifice diameter and position and thickness of the anhydrite-dominated stage I wall are not just a result of initial mixing between the plume and ambient sea water³, but are also controlled by the dynamics of fluid flow in the chimney channel after wall emplacement.

This experiment demonstrates that *in situ* monitoring of vent phenomena is possible. The extent to which the solid collar wall and coupons influence mixing and/or fluid flow in the chimney

FIG. 2 a, Average temperatures for 12-h intervals measured over 46 days. Maximum temperatures recorded by TC03, TC04, TC05, TC07, TC08 and TC10 were 372, 405, 372, 381, 365 and 375°C respectively. The remaining thermocouples in the test collar recorded maximum temperatures of $353 \pm 2^\circ \text{C}$, and TC02 recorded $353 \pm 2^\circ \text{C}$ for the duration of the experiment. b, Average temperatures for 30-min intervals measured during the initial wall-building stage. Numbers refer to thermocouples. Immediately outside the test collar, temperatures varied between 6 and 16°C for the duration of the experiment (TC12 and TC13), and at the platform containing the sampling and storage system the temperature varied from 2.5 to 3.2°C . Temperatures were measured using ungrounded type K (chromel-alumel) thermocouples with error limits of $\pm 2.2^\circ \text{C}$ up to 277°C and $\pm 0.75\%$ for temperatures between 277 and $1,260^\circ \text{C}$. The electronics package and reference RTD were moored 1 m away from the active vent, housed in a 1.77-cm-thick PVC case located at the end of a 6-m-long cable. Calibration before deployment consisted of placing the electrical-junction box in a thermostatically controlled freezer and the test collar and thermocouples



in a vessel of boiling water. During the 24-hour test the RTD and all thermocouples performed within the expected error limits. Recalibration after the experiment was not done because it would have required dissolution of the chimney wall.

channel can be minimized in future experiments by modifying the instrument—for example, by eliminating the coupons and suspending a set of thermocouples above the collar. Longer experiments can be coupled with time-lapse camera studies^{20,21} so that wall position and upward growth rates can be discerned. This technique can be used to investigate transport and chemical reaction across steep thermal and chemical gradients, the time-scale and long-term stability of sea-floor vent structures, and the stability and range of temperatures exhibited in hydrothermal systems. □

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- Haymon, R. M. *Nature* **301**, 695–698 (1983).
- Goldfarb, M. S. *et al. Econ. Geol. Monogr.* **5**, 184–197 (1983).
- Turner, J. S. & Campbell, I. H. *Earth planet. Sci. Lett.* **82**, 36–48 (1987).
- Campbell, A. C. *et al. Nature* **335**, 514–519 (1988).
- Campbell, A. C. *et al. J. geophys. Res.* **93**, 4537–4549 (1988).
- Delaney, J. R. *et al. Eos* **70**, 1163 (1989).
- Delaney, J. R., McDuff, R. E. & Lupton, J. E. *Eos* **65**, 973 (1984).
- Seyfried, W. E. Jr. *Rev. Earth planet. Sci.* **15**, 317–335 (1987).
- Berndt, M. E., Seyfried, W. E. Jr & Janeky, D. R. *Geochim. cosmochim. Acta* **53**, 2283–2300 (1989).
- Karsten, J. *et al. Eos* **65**, 1111 (1984).
- Tivey, M. K. & Delaney, J. R. *Earth planet. Sci. Lett.* **77**, 303–317 (1986).
- Bischoff, J. L. & Rosenbauer, R. J. *Am. J. Sci.* **285**, 725–763 (1985).
- Hekinian, R. *et al. Mar. geophys. Res.* **6**, 1–14 (1983).
- Styr, M. M. *et al. Earth planet. Sci. Lett.* **53**, 382–390 (1981).
- Freeze, R. A. & Cherry, J. A. *Groundwater* (Prentice-Hall, Englewood Cliffs, 1979).
- Robie, R. A., Hemingway, B. S. & Fisher, J. R. *U.S. Geol. Survey Bull.* **1452**, 1–456 (1979).
- Holland, H. D. *The Chemistry of the Atmosphere and Oceans* (Wiley, New York, 1978).
- Yusofova, V. D. *et al. Desalination* **25**, 269–280 (1978).
- Converse, D. R., Holland, H. D. & Edmond, J. M. *Earth planet. Sci. Lett.* **69**, 159–175 (1984).
- Johnson, H. P. & Tunncliffe, V. *Geophys. Res. Lett.* **12**, 685–688 (1985).
- Johnson, H. P. & Tunncliffe, V. *Eos* **67**, 1283 (1986).
- Miller, V. W. *Oceans '89 Conf. Proc. Vol. 1*, 26–31 (IEEE, Piscataway, 1989).
- Oudin, E. *Mar. Mining* **4**, 39–72 (1983).
- Fouquet, Y. *et al. Mar. Geology* **84**, 145–178 (1988).
- Graham, U., Bluth, G. & Ohmoto, H. *Can. Mineral.* **26**, 487–504 (1988).
- Haymon, R. M. & Kastner, M. *Am. Mineral.* **71**, 819–825 (1986).
- Zierenberg, R. A., Shanks, W. C. III & Bischoff, J. L. *Geol. Soc. Am. Bull.* **95**, 922–929 (1984).
- Haymon, R. M. & Kastner, M. *Earth planet. Sci. Lett.* **53**, 363–381 (1981).

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Eclogitic diamonds of Proterozoic age from Cretaceous kimberlites

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DIAMONDS with eclogitic inclusions ('eclogitic diamonds') from the Premier kimberlite and Argyle lamproite were previously shown to be of Proterozoic age^{1–3} and distinct in origin from peridotitic diamonds of Archaean age⁴ and deep-continental-mantle (tectospheric⁵) provenance. The Premier and Argyle host rocks are themselves of Proterozoic age, suggesting a link between eclogitic diamond formation and kimberlite or lamproite magmatism¹. Here we test this link by examining eclogitic diamonds from younger southern African kimberlites. Eclogitic garnet and clinopyroxene inclusions in Orapa and Finsch diamonds yield Sm–Nd isochron ages of 990 and 1,580 Myr respectively, compared with host kimberlite emplacement ages of ~100 Myr. These eclogitic diamond ages are again Proterozoic, and distinct from the ~3,200-Myr age of Finsch peridotitic diamonds⁴. Thus peridotitic and eclogitic diamonds from the same locality are evidently both xenocrysts, having been stored in the deep continental mantle beneath the Kaapvaal craton for extended periods before being sampled by kimberlite. Such mantle storage may not be detected by the

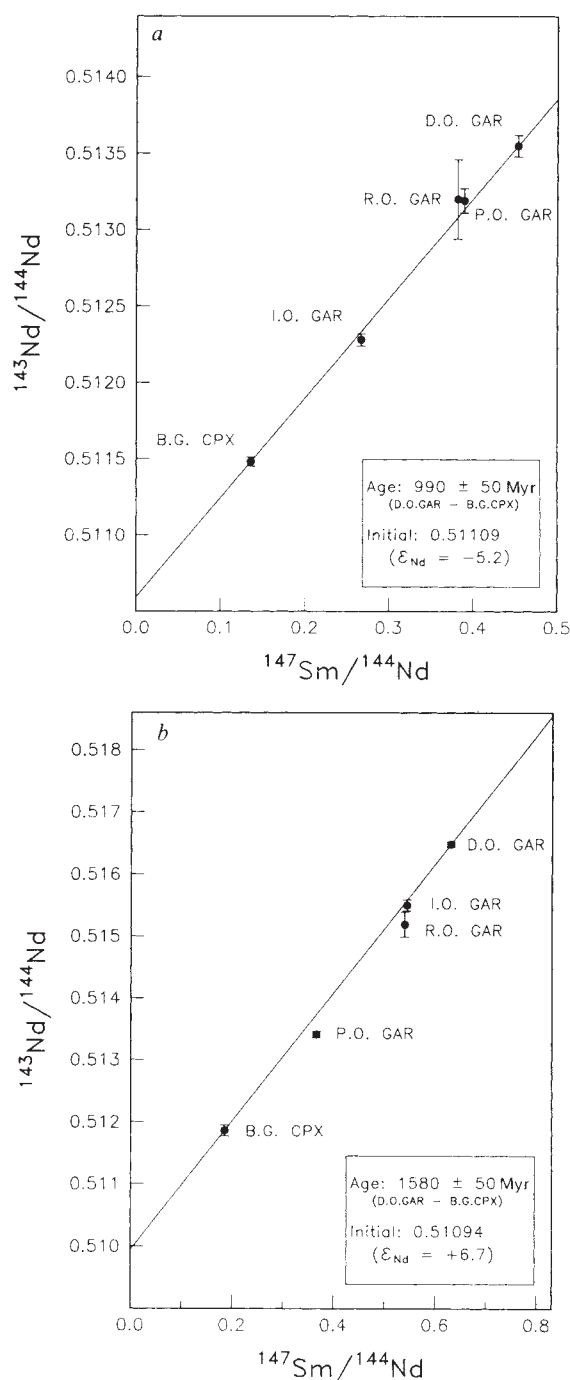


FIG. 1 Sm–Nd isochron diagrams for eclogitic garnet and clinopyroxene inclusions in Orapa (a) and Finsch (b) diamonds with sample abbreviations as in Table 1. The ages are calculated for the deep-orange garnet/bluish-green clinopyroxene pairs, using $\lambda_{\text{Sm}} = 6.54 \times 10^{-12} \text{ yr}^{-1}$, with assigned errors derived from maximum and minimum slopes allowed by $2\sigma_{\text{mean}}$ measurement errors.

⁴⁰Ar–³⁹Ar laser-probe dating technique as applied to Premier diamonds^{2,3}.

The Premier kimberlite, where the ⁴⁰Ar–³⁹Ar laser-probe dating technique was first tested on eclogitic diamond inclusions^{2,3}, is an unusual case in which similar diamond crystallization and kimberlite emplacement ages cannot be resolved by current dating techniques. Nevertheless, these diamonds have nitrogen aggregation characteristics requiring a minimum 1–10 Myr period of mantle storage (within the errors of the age determinations) and implying a xenocrystal relationship to the host kimberlite itself¹. R. Burgess (personal communication) has recently

TABLE 1 Isotopic composition of eclogitic garnet and clinopyroxene inclusions

	Wt (mg)	Rb	Sr	$^{87}\text{Rb}/^{86}\text{Sr}$	$^{87}\text{Sr}/^{86}\text{Sr}$	$^{87}\text{Sr}/^{86}\text{Sr}_i$	Sm	Nd	$^{147}\text{Sm}/^{144}\text{Nd}$	$^{143}\text{Nd}/^{144}\text{Nd}$	$^{143}\text{Nd}/^{144}\text{Nd}_i$
Orapa*											
d.o. gar	3.04	0.002	3.49	0.0020	0.70819 (2)	0.70816	2.129	2.840	0.4533	0.51405 (7)	0.51109
i.o. gar	2.17	0.009	7.02	0.0036	0.70775 (3)	0.70770	2.328	5.273	0.2669	0.51278 (4)	0.51104
p.o. gar	0.59	0.004	4.89	0.0022	0.70812 (4)	0.70809	1.671	2.595	0.3893	0.51369 (8)	0.51115
r.o. gar	0.71	0.012	2.23	0.016	0.71068 (5)	0.71045	2.393	3.793	0.3815	0.51370 (26)	0.51121
b.g. cpx	1.78	0.203	356.6	0.0017	0.70608 (3)	0.70606	1.229	5.458	0.1361	0.51198 (3)	0.51109
Finsch†											
d.o. gar	2.17	0.002	3.62	0.0015	0.70512 (5)	0.70509	1.917	1.839	0.6308	0.51748 (4)	0.51094
i.o. gar	1.11	0.005	4.09	0.0038	0.70661 (5)	0.70652	2.068	2.302	0.5435	0.51650 (9)	0.51086
p.o. gar	0.85	0.007	7.79	0.0026	0.70523 (3)	0.70517	2.148	3.551	0.3658	0.51441 (5)	0.51062
r.o. gar	0.31	0.015	1.34	0.033	0.71161 (24)	0.71086	0.723	0.811	0.539	0.51619 (20)	0.51060
b.g. cpx	0.48	0.214	111.6	0.0055	0.70474 (2)	0.70462	0.973	3.172	0.1854	0.51286 (9)	0.51094
F11‡	1.10	0.001	7.09	0.0002	0.70501 (3)	0.70501					

Concentrations in p.p.m. ($\mu\text{g per g}$), after correction for minimum applicable blanks: Rb, 3 pg; Sr, 4 pg; Sm, 0.6 pg; Nd, 1.5 pg. Estimated analytical uncertainties in concentrations (ignoring small-sample weighing errors) are indicated by the number of significant digits. Present-day $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{143}\text{Nd}/^{144}\text{Nd}$ ratios are normalized to 0.70800 and 0.51264 for standards E & A SrCO_3 and BCR-1, respectively. In-run precision is indicated by the errors ($2\sigma_{\text{mean}}$) in the least-significant digits given in parentheses. Initial ratios (denoted by subscript i) calculated for Orapa and Finsch diamond crystallization ages of 990 and 1,580 Myr, respectively, using decay constants $\lambda_{\text{Rb}} = 1.42 \times 10^{-11} \text{ yr}^{-1}$ and $\lambda_{\text{Sm}} = 6.54 \times 10^{-12} \text{ yr}^{-1}$. Inclusion-bearing diamonds were selected from the -6 +5 diamond sieve fraction (1.8–2.2 mm diameter) of general mine production.

* Orapa composites: ~160 deep-orange garnets (d.o. gar), 110 intermediate-orange garnets (i.o. gar), 60 pale-orange garnets (p.o. gar) and 80 reddish-orange garnets (r.o. gar) (average weight 16 μg); ~220 bluish-green clinopyroxenes (b.g. cpx) (average weight 8 μg).

† Finsch composites: ~150 d.o. gar, 80 i.o. gar, 130 p.o. gar and 60 r.o. gar (average weight 11 μg); ~90 b.g. cpx (average weight 5 μg).

‡ Single large d.o. gar successfully analysed for Rb–Sr only in 1985.

obtained ^{40}Ar – ^{39}Ar ages for eclogitic clinopyroxene inclusions in Orapa diamonds approaching that of the host kimberlite. Whereas diamond encapsulation precludes diffusive exchange of elemental species between inclusions that are not touching, these species are able to diffuse through the inclusion minerals themselves at mantle temperatures and pressures. Further, although cations such as Sm, Nd, Rb, Sr and K remain within the crystal structures of the inclusion minerals, the noble gas Ar seems to be able to accumulate at inclusion/diamond interfaces, effectively separating radiogenic Ar from the parent K and resetting the K–Ar clock within clinopyroxene until removal to the surface. This technique may therefore give ages indicative more of the kimberlite emplacement event than of the diamond crystallization event.

The Orapa kimberlite is located in the Archaean Limpopo Mobile Belt in northeastern Botswana. A zircon U–Pb age of 93 Myr provides the most reliable estimate of emplacement age⁶, which is Cretaceous and consistent with the preservation of crater facies deposits⁷. The Orapa locality was chosen for this study because of its relative abundance of diamonds containing eclogitic inclusions. The eclogitic and lesser websteritic assemblages of orange garnet, bluish-green clinopyroxene, sulphides and orthopyroxene together comprise >85% of inclusions in Orapa diamonds⁸.

The Finsch kimberlite is located in the southwestern interior of the Archaean Kaapvaal craton. In the absence of zircons, a phlogopite Rb–Sr age of 118 Myr provides the most reliable estimate of emplacement age⁹. The Finsch locality was chosen because of the Archaean age of its dominant peridotitic-inclusion-bearing diamonds⁴ and because of a parallel study of individual large diamonds containing eclogitic garnet inclusions, by Smith *et al.* (ref. 10 and manuscript in preparation). The eclogitic assemblage of orange garnet, bluish-green clinopyroxene and sulphides accounts for <2% of inclusions in Finsch diamonds¹¹, so extensive sorting was required to collect sufficient material for analysis.

Small-sample preparation techniques and mass spectrometric facilities for the determination of Sm, Nd, Rb and Sr concentrations and isotope ratios (Table 1) were the same as those used in ref. 1. Here, further categories of garnet were identified on the basis of subtle variations in colour. Major-element analyses of single specimens of each of the resultant deep-, intermediate-, pale- and reddish-orange garnet and bluish-green clinopyroxene categories are reported in Table 2. By comparison with world-wide occurrences¹², these are all clearly eclogitic with the possible exception of the Orapa reddish-orange garnet, which displays a websteritic character⁸. This colour characterization is used for convenience in separating

components with a spread of Sm/Nd ratios between those of deep-orange garnet and bluish-green clinopyroxene, which constitute a cogenetic pair in rare biminerally-inclusion-bearing diamonds (not used in this study)¹. Otherwise, we used the same analytical strategies and precautions with respect to composites as in ref. 1. In particular, Rb/Sr ratios for both garnet and clinopyroxene are extremely low, resulting in barely significant increases in $^{87}\text{Sr}/^{86}\text{Sr}$ ratio on a 1,000-Myr timescale. Thus, $^{87}\text{Sr}/^{86}\text{Sr}$ ratios can be used to examine the cogeneticity of garnet and clinopyroxene inclusions from separate diamonds, which have been paired to derive ages from Sm–Nd isochrons.

The Orapa deep-orange garnet/clinopyroxene pair defines a two-point Sm–Nd isochron age of 990 ± 50 Myr (Fig. 1a), where the error is derived from the maximum and minimum slopes allowed by $2\sigma_{\text{mean}}$ measurement errors (Table 1). The intermediate-, pale- and reddish-orange garnets with intermediate Sm/Nd ratios also lie on the isochron, within the errors. Regression analysis of the complete data set is not warranted, however, because the initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of the garnets (0.7077–0.7105) are all higher than that of the clinopyroxene (0.7061) as also shown on a present-day Sr isotope mixing diagram (Fig. 2). Ideally, the deep-orange garnet and the clinopyroxene should have the same $^{87}\text{Sr}/^{86}\text{Sr}$ ratio if they are cogenetic (as in diamonds with biminerally inclusions¹) but the actual datum for this composite in Fig. 2 indicates that the colour categories are unreliable and/or were not well separated during sample preparation. The reddish-orange garnet has the highest $^{87}\text{Sr}/^{86}\text{Sr}$ ratio, making it the other obvious endmember component. Nevertheless, the shape of the Orapa data field in Fig. 2 rules out simple two-component mixing as an explanation for the linear relationship in Fig. 1a.

Similarly, the Finsch deep-orange garnet/clinopyroxene pair defines a two-point Sm–Nd isochron age of $1,580 \pm 50$ Myr (Fig. 1b). The intermediate-, pale- and reddish-orange garnets again have intermediate Sm/Nd ratios but the latter two lie well below the isochron. The initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of the garnets (0.7050–0.7109), including that for a single large deep-orange garnet inclusion (F11 in Table 1), are again higher than that of the clinopyroxene (0.7046) but in this case the deep-orange garnet and clinopyroxene have reasonably similar values (Fig. 2). Again, the reddish-orange garnet has the highest $^{87}\text{Sr}/^{86}\text{Sr}$ ratio. Further, a single large Finsch eclogitic garnet inclusion analysed by Smith *et al.*¹⁰ lies on the 1,580-Myr isochron in Fig. 1b (at 0.702, 0.51818(5)) albeit with a slightly lower $^{87}\text{Sr}/^{86}\text{Sr}$ ratio (0.7041).

The $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of the different garnet composites indicate the presence of several different components in the eclogitic inclusion assemblage at both localities. The two extremes are

TABLE 2 Major-element composition of eclogitic garnet and clinopyroxene inclusions

	Orapa					Finsch				
	d.o. gar	i.o. gar	p.o. gar	r.o. gar	b.g. cpx	d.o. gar	i.o. gar	p.o. gar	r.o. gar	b.g. cpx
SiO ₂	39.49	40.41	39.80	41.01	55.40	38.45	39.42	40.93	39.01	55.80
TiO ₂	0.66	0.44	0.55	0.51	0.99	0.37	0.35	0.12	0.16	0.51
Al ₂ O ₃	22.17	23.17	22.75	21.20	9.68	21.78	22.82	22.78	21.42	9.98
Cr ₂ O ₃	0.08	0.05	0.07	2.27	0.15	0.05	0.04	0.31	0.08	0.11
FeO	18.84	16.27	17.75	13.79	7.79	25.62	17.53	17.90	25.56	5.27
MnO	0.42	0.36	0.37	0.46	0.11	1.16	0.37	1.16	1.40	—
MgO	9.53	12.38	9.65	15.46	10.52	8.20	8.65	14.97	8.27	10.00
CaO	8.90	7.36	9.10	4.92	9.09	4.63	11.31	2.39	4.03	11.84
Na ₂ O	0.18	0.19	0.18	0.04	5.60	0.16	0.17	0.11	0.14	5.83
K ₂ O	—	—	—	—	0.37	—	—	—	—	0.07
Total	100.27	100.63	100.22	99.66	99.70	100.42	100.66	100.67	100.07	99.41

Concentrations (wt%) were determined by electron microprobe.

represented by the 'pure' deep-orange garnet/clinopyroxene pair and the reddish-orange garnet. Nevertheless, the Sm-Nd isotope data conform reasonably well to isochron relationships implying a more restricted range of initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratios than the range of initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in each case. This can be understood in terms of a trace-element-enriched (high Rb/Sr, low Sm/Nd) character for the precursor of the reddish-orange garnet, resulting in radiogenic initial $^{87}\text{Sr}/^{86}\text{Sr}$ and nonradiogenic initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratios for such components. Thus, the isochron relationships in Fig. 1 seem to have age significance (rather than being mixed lines) and the corresponding initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratios indicate a dominantly enriched precursor at Orapa ($\epsilon_{\text{Nd}} = -5$; $\epsilon_{\text{Nd}} = \{[(^{143}\text{Nd}/^{144}\text{Nd})_{\text{sample}} / (^{143}\text{Nd}/^{144}\text{Nd})_{\text{bulk Earth}}] - 1\} \times 10^4$) and a depleted precursor at Finsch ($\epsilon_{\text{Nd}} = +7$). This difference is also reflected in the carbon isotopic composition of eclogitic diamonds which at Finsch have $\delta^{13}\text{C}$ values of -3 to -8% (ref. 13; $\delta^{13}\text{C}(\%) = [(^{13}\text{C}/^{12}\text{C})_{\text{sample}} / (^{13}\text{C}/^{12}\text{C})_{\text{standard}}] - 1$, PDB standard consistent with a depleted upper-mantle carbon source, and at Orapa values of -3 to -23% (P. Deines *et al.*, manuscript in preparation), which may indicate the involvement of recycled crustal carbon (M. B. Kirkley *et al.*, manuscript in preparation).

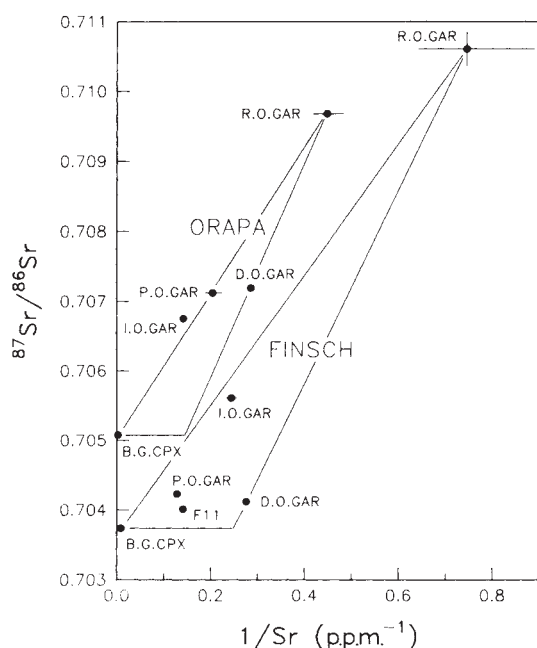


FIG. 2 Sr isotope mixing diagram with present-day $^{87}\text{Sr}/^{86}\text{Sr}$ plotted against reciprocal Sr concentration for eclogitic inclusions in Orapa and Finsch diamonds. Errors in Sr concentration are dominated by a nominal small-sample weighing error of ± 0.05 mg. Mixing curves are straight lines and nominal mixing envelopes for Orapa and Finsch inclusion composites are indicated although no direct mixing of garnet and clinopyroxene is implied.

Thus there is clear evidence that eclogitic diamond formation involved at least two enriched components at Orapa and both depleted and enriched components at Finsch. Also, the precursor Nd and C isotope signatures of eclogitic diamonds from Finsch and Orapa resemble those from Premier and Argyle¹, respectively. In contrast to peridotitic diamond formation, which can be correlated with early Archaean komatiite magmatism and continental mantle stabilization⁴, eclogitic diamond formation has yet to be correlated with regional tectonothermal events, although subduction is a distinct possibility. Further, the crystallization ages of peridotitic and eclogitic diamonds from Finsch differ by more than 1,500 Myr, and their precursors have contrasting enriched and depleted isotope signatures. The radiogenic Sr component evident in reddish-orange garnet inclusions in eclogitic diamonds may be derived from subducted crust and/or the enriched garnet harzburgite host assemblages of peridotitic diamonds stored in tectospheric mantle since the Archaean⁴.

More generally, trace-element-depleted components (characterized by positive ϵ_{Nd} values) are derived from asthenospheric mantle (the source of mid-ocean-ridge basalts) whereas trace-element-enriched components (particularly those with negative ϵ_{Nd} values) are derived from continental tectospheric mantle and/or continental crust. As a corollary, enriched components with negative ϵ_{Nd} values are difficult to obtain directly from metamorphosed ocean crust. Finally, the combination of components in the above-mentioned eclogitic diamonds must be assembled and stored in stable upper mantle at depths of 150–200 km (ref. 14) on a 1,000-Myr timescale. Such eclogitic diamonds therefore most plausibly reflect local entrapment, mixing and crystallization of asthenosphere-derived magmas (which may include crustal components recycled by subduction) in tectospheric mantle beneath the Kaapvaal craton during the Proterozoic. This ancient continental-mantle keel subsequently survived convective disruption leaving original peridotitic and later eclogitic diamonds available for sampling by kimberlite in the Cretaceous. □

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- Richardson, S. H. *Nature* **322**, 623–626 (1986).
- Burgess, R., Turner, G., Laurenzi, M. & Harris, J. W. *Earth planet. Sci. Lett.* **94**, 22–28 (1989).
- Phillips, D., Onstott, T. C. & Harris, J. W. *Nature* **340**, 460–462 (1989).
- Richardson, S. H., Gurney, J. J., Erlank, A. J. & Harris, J. W. *Nature* **310**, 198–202 (1984).
- Jordan, T. H. in *Oceanic and Continental Lithosphere: Similarities and Differences* (eds Menzies, M. A. & Cox, K. G.) 11–37 (Oxford University Press, 1988).
- Davies, G. L. *Yb. Carnegie Inst. Wash.* **76**, 631–635 (1977).
- Hawthorne, J. B. *Phys. Chem. Earth* **9**, 1–15 (1975).
- Gurney, J. J., Harris, J. W. & Rickard, R. S. in *Kimberlites II: The Mantle and Crust-Mantle Relationships* (ed. Kornprobst, J.) 3–10 (Elsevier, Amsterdam, 1984).
- Smith, C. B., Allsopp, H. L., Kramers, J. D., Hutchinson, G. & Roddick, J. C. *Trans. Geol. Soc. S. Afr.* **88**, 249–266 (1985).
- Smith, C. B. *et al.* in *Kimberlites and Related Rocks* (eds Ross, J. *et al.*) 853–863 (Blackwell, Melbourne, 1989).
- Gurney, J. J., Harris, J. W. & Rickard, R. S. in *Kimberlites, Diatremes and Diamonds* (eds Boyd, F. R. & Meyer, H. O. A.) 1–15 (Am. geophys. Un., Washington, DC, 1979).
- Meyer, H. O. A. in *Mantle Xenoliths* (ed. Nixon, P. H.) 501–522 (Wiley, Chichester, 1987).
- Deines, P., Gurney, J. J. & Harris, J. W. *Geochim. cosmochim. Acta* **48**, 325–342 (1984).
- Boyd, F. R., Gurney, J. J. & Richardson, S. H. *Nature* **315**, 387–389 (1985).

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Involvement of the oxygen minimum in benthic zonation on a deep seamount

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LOW oxygen concentration in the seawater column reduces the abundance of midwater consumer populations^{1,2}, which can enhance the supply of undegraded organic matter reaching the benthos. Low oxygen concentration in the water at the bottom can exclude most tolerant species from benthic habitats³⁻⁵. The interception of the seafloor with pronounced oxygen-minimum zones can produce steep gradients in benthic assemblages. We now present evidence for this interaction on Volcano 7, an oceanic seamount penetrating the oxygen-minimum zone in the eastern tropical Pacific. Submersible observations revealed only a few benthic species at the summit (730–750 m), where oxygen levels were lowest. Just tens of metres below, megafaunal and macrofaunal abundances were extremely high. Sediment organic carbon, a benthic food indicator, was unusually high. We hypothesize that a dynamic low-oxygen interface physiologically restricts benthos on the upper summit, that the enriched sediment is a result of reduced consumption and degradation of sinking material in the oxygen-minimum zone, and that this high benthic food level supports the unusually high benthic abundance. Sharp benthic zonation associated with oxygen concentrations may also be preserved in the palaeoceanographic record^{4,6}.

Volcano 7, an inactive seamount⁷ at 13° 23' N, 102° 27' W, of

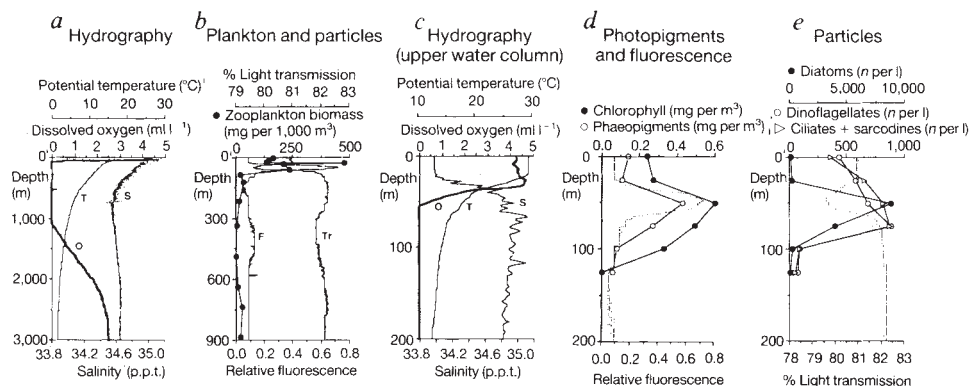
20-km diameter, rises from a depth of 3,400 m to 730 m. Its benthic and pelagic ecology were studied with submersible and shipboard sampling (including conductivity temperature depth (CTD) hydrocasts, water samples, box cores, photography, plankton tows, current meters and sediment traps) on a cruise in November 1988. Benthic data from June 1984 are also discussed.

The eastern tropical Pacific is characterized by a shallow mixed layer and a pronounced oxygen minimum⁸. At the Volcano 7 site, the mixed layer was 22 m deep, and the base of the euphotic zone was at 85 m. Sharp peaks of fluorescence, particles, chlorophyll *a*, phytoplankton, protozoans and zooplankton occurred just below the mixed layer (Fig. 1). The core of the oxygen minimum zone (oxygen <0.5 ml per litre of seawater) occurred from median depths of 72–1302 m, and the lowest oxygen (<0.1 ml per litre) from median depths of 288–1,077 m (Fig. 1a). Thus the base of the euphotic zone was within the oxygen-minimum zone, and particles sinking out of the euphotic zone immediately entered a broad region of low oxygen and reduced plankton abundance.

There is evidence that undegraded surface-derived material reached the seamount summit. Bottom-moored particle interceptor traps^{9,10} collected intact microplanktonic organisms common in surface waters. Excess Pb-210 activity profiles¹¹ indicated high sedimentation rates on the summit of 0.3–0.7 mm yr⁻¹. Sediment organic carbon (up to 3.9%), organic nitrogen (up to 0.55%), chlorophyll *a* (up to 25 µg g⁻¹) and sediment bacterial abundances were remarkably high on the summit, especially at depths <770 m (Fig. 2). Here, C/N ratios were near 7.0 (Fig. 2c), indicating the presence of fresh organic matter¹². Green flocculent material was present on the bottom in both November 1988 and June 1984. The supply of undegraded organic material to the benthos may be more continuous in this region of reduced seasonality¹³ than in higher latitudes, where benthic fluxes are strongly seasonal^{14,15}.

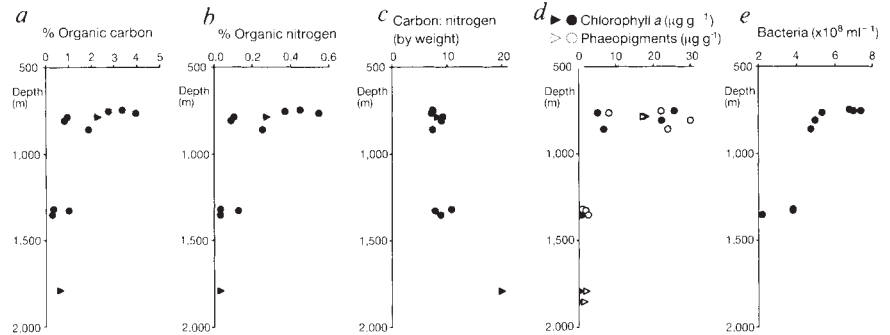
Strong megafaunal zonation was observed on the summit of the seamount (Figs 3 and 4). The uppermost summit (<750 m) was visibly depauperate (Fig. 3a and b). Gradual increases in megafauna occurred in a transition zone from 750–770 m.

FIG. 1 Vertical profiles through the water column of physical, biological and chemical parameters. The sharp shallow thermocline is also an abrupt transition from oxygenated mixed-layer water to the very low-oxygen water in the oxygen-minimum zone. Concentrations of organisms (day and night), photopigments and particles peak sharply within the thermocline, then drop abruptly to low levels in the oxygen-minimum zone. Temperature, salinity and dissolved oxygen in the water column were measured with a SBE 19 Seacat CTD Profiler. Dissolved oxygen concentrations were calibrated by the Winkler titration method²⁶ on individual water samples from hydrocasts, and the oxygen values from the CTD electrode were transformed on the basis of a linear equation relating the two sets of measurements. Thirty-one CTD casts were made at locations on the seamount and along north-south and east-west transects extending up to 40 km from the summit. Data from the downcast of a representative CTD profile above the seamount are shown in *a* (whole water column) and *c* (upper 200 m detail). Zooplankton were obtained in 15 vertically stratified tows (nine samples per tow) at various locations on and near the seamount by a 1 m² MOCNESS (ref. 27) multiple opening and closing plankton net system using 335-µm mesh nets. Wet-weight zooplankton biomasses from a night-time tow series above the seamount are plotted in *b*. Relative fluorescence was measured by a Sea Tech *in situ* fluorometer and % light transmission by a Sea Tech 25-cm beam transmissometer, both attached



to the MOCNESS. Representative profiles are shown in *b* and *e*. Fluorescence and light transmission data shown in *b* were smoothed with a five-point running mean. Fluorescence data in *d* were smoothed with a three-point running mean. Chlorophyll *a* and phaeophytin values (*d*), from water overlying the seamount, are means of two or three water samples per depth with two 500-ml aliquots per sample. Measurements were made fluorometrically²⁸ on water collected with Niskin bottles and filtered onto GFF filters. Abundances of diatoms, dinoflagellates and ciliate plus sardocodine protozoa (*e*) are means of three counts from water overlying the seamount (*n* per litre). Aliquots of 100 ml were drawn from Niskin bottles, preserved with Lugol's iodine, settled in settling chambers, and the entire sample was counted under an inverted microscope (magnification, ×170).

FIG. 2 Sediment organic matter properties. 1984 data, $\blacktriangle$; 1988 data, $\bullet$. Analyses of organic carbon (a), nitrogen (b), C/N (c), and chlorophyll *a* ($\blacktriangle$, $\bullet$) and phaeopigments ($\triangle$, $\circ$) (d) were made on sediments collected by the submersible in tube cores and frozen at -70°C . Organic C and N were analysed on the 0–2 cm vertical fraction. Sediments were homogenized, dried and ground, inorganic carbon was leached with 10% phosphoric acid for 24 h, then sediments were rinsed with organic-free distilled water, centrifuged, and the supernatant was decanted. The leaching process was repeated three times and remaining sediments were freeze-dried, weighed, ground and analysed on a Carlo Erba elemental analyser. Sediment chlorophyll *a* and phaeopigments were determined for 3 g sediment from the upper 1 cm using the spectrophotometric method²⁸ modified for sediments. Immediately on retrieval of tube cores, sediment bacteria (e) (1 ml from the top 1 cm) were sampled and preserved in 9 ml of 2.5% glutaraldehyde. Counts were made in the laboratory within two months of collection, using acridine-orange staining and epifluorescence microscopy²⁹.



There were significant differences (by analysis of variance) between upper (<770 m) and lower (770–900 m) summit locations for organic C ($P=0.015$) and N ($P=0.012$). Differences between summit (combined) and flank values were observed for organic C ($P=0.027$), organic N ($P=0.023$), chlorophyll *a* ($P=0.009$), phaeopigments ($P=0.001$) and bacteria ($P=0.004$).

Extraordinarily high densities (up to 19 individuals per m^2 in patches) occurred from 780–1,000 m, with maxima from 800–810 m (Figs 3c, d and 4a–f). Below 1,000 m, average megafaunal abundances (excluding xenophyophores) never exceeded 0.5 per m^2 . This zonation did not correspond to the distribution of hard and soft substrates, and no hydrothermal activity was observed.

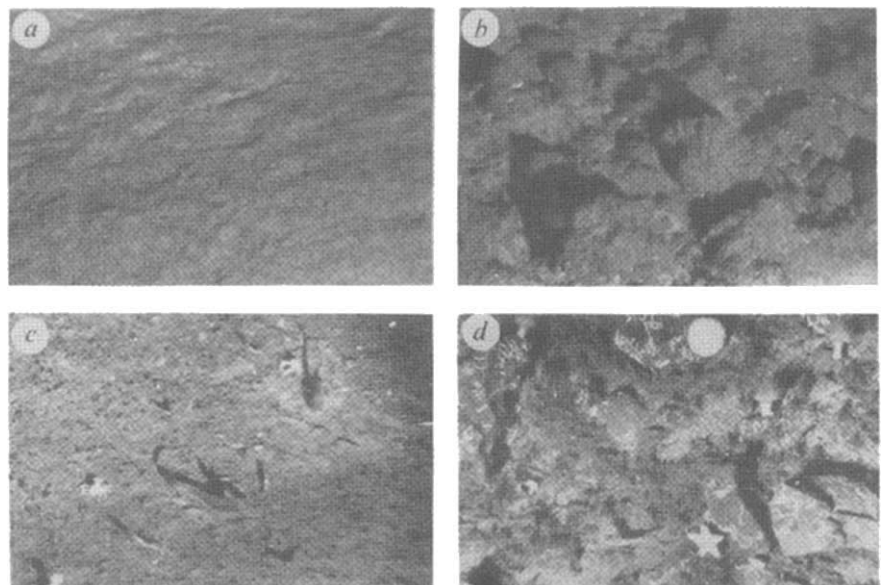
Abundance patterns of infauna, obtained in Ekman-style box cores, differed with size. Macrofauna ($>300\ \mu\text{m}$) were rare above 750 m, with only low-oxygen-tolerant forms^{16,17} present. Macrofauna increased greatly from the uppermost zone to 800–1,000 m (8,444 per m^2), then declined downslope (Fig. 4g). Meiofauna (smaller infauna), which are more tolerant than macrofauna to hypoxia¹⁸, were most abundant at depths <750 m (202, 100 per m^2 , $n=2$) (Fig. 4h). The nematode to harpacticoid copepod ratio, an indicator of oxygen stress¹⁹, was 546:1 on the uppermost summit and declined to 1.3:1 on the flank (Fig. 4i).

Oxygen in near-bottom water samples obtained by submersible was 0.08–0.09 ml per litre ($n=2,733$ –746 m) in the uppermost summit region of low megafaunal and macrofaunal abund-

ance and 0.11–0.16 ml per litre ($n=3,806$ –815 m) in the region of highest abundance. In five of six seamount CTD casts, oxygen was lower at 730 m than at 800 m, but there was considerable overlap in the concentration range measured at these two depths. Diurnal and semidiurnal temperature fluctuations (0.5°C) occurred at the summit, indicating that internal tides were displacing water masses. This suggests that benthic organisms on the summit were exposed to periodically varying oxygen rather than to constant oxygen concentrations. Physiological exclusion of benthos at dynamic oxygen interfaces may therefore be a function of amplitude and temporal variation as well as absolute oxygen concentrations.

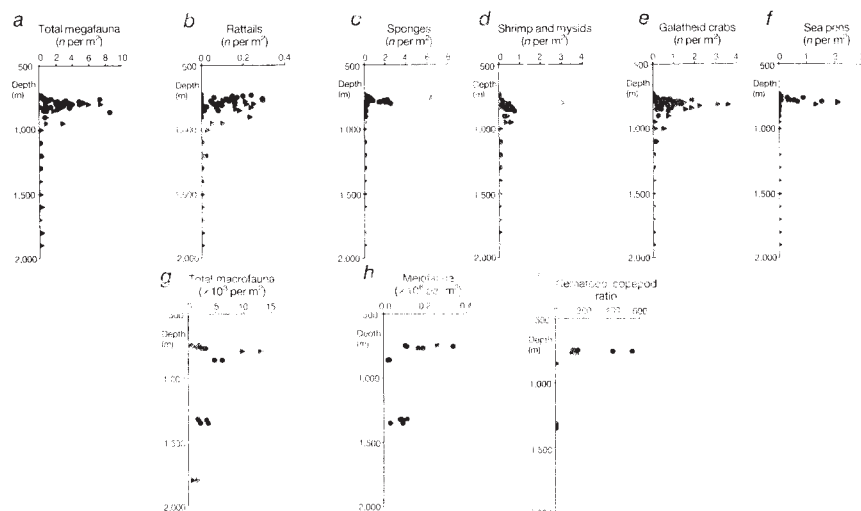
Very low plankton abundances are associated with oxygen minimum zones in the water column in parts of the Arabian Sea and the eastern Pacific, but only where oxygen is less than about 0.15 ml per litre throughout a broad depth interval^{1,2}, similar to the Volcano 7 region. At locations with more oxygen, plankton biomass may not be reduced, although distributions may be altered^{1,20–22}. For midwater crustaceans, an oxygen concentration of 0.13 ml per litre is a critical lower oxygen limit for

FIG. 3 Representative photographs of the upper summit (a, b; 744 and 734 m respectively) and lower summit (c, d; 770 and 780 m respectively). a and c show foraminiferal sand substrate, whereas b and d show rocky and mixed substrate. The only megafauna on the upper summit (a, b) were occasional rattail fish (*Nezumia liolepis*, shown in c and d but not in upper summit photographs) and solitary sessile coelenterates under rocks (White dots in b). Rock surfaces appear fuzzy (b, d) because of dense mats of an unidentified tube-building protozoan. Dark green detrital material was visible on rocks and sediments. Upper summit (a) meiofauna included large numbers of nematodes. The most abundant macrofauna were an aplacophoran (family Lepidomeniidae), a paragonid polychaete (*Cirrophorus tyra*), a dorvilleid polychaete (*Protodurvillea* sp.) and a few pogonophorans. A variety of abundant megafauna occurred on both rocks and sediments on the lower summit (b, d). Rattail fish (*N. liolepis*), galatheid crabs (*Munidopsis* c.f. *hystrix*), shrimp, starfish, sponges, anemones, ophiuroids and serpulid worm tubes (*Hyalopomatus marenzelleri*) are some of the organisms in c and d. Here, dark green or green-grey flocculent material occurred in depressions. Infaunal densities were high for sand substrates ($>8,000$ per m^2), and dominant infauna included cirratulid, sabellid and dorvilleid polychaetes and burrowing anemones. These photographs were taken with a



hand-held 35-mm camera from inside the *Alvin* submersible and show an area of 2–3 m^2 . For scale, the galatheid crabs are about 5 cm from claw tip to tail.

FIG. 4 Benthic faunal densities on the summit and flank as a function of water depth. 1984 data, $\blacktriangle$; 1988 data, $\bullet$. Megafaunal abundances were quantified from 1988 *Alvin* photographs (external hull-mounted camera) using a perspective grid to calculate surface area, and from 1984 *Angus* camera sled photographs, in which area was calculated as 1.25 sled altitude $\times$ 1.8 sled altitude. Mean faunal densities are shown for 10-m depth intervals 730–850 m, for 50-m intervals 850–1,000 m, and for 100-m intervals 1,000–2,000 m. Data from photographs taken on different parts of the seamount (at comparable depths) are plotted separately. Maximum densities of major taxa (1984 photographs) were 4.3 per m^2 for sponges, 9.7 per m^2 for galatheid crabs, 7.3 per m^2 for combined shrimp and mysids, 1.8 per m^2 for rattail fishes and 4.9 per m^2 for sea pens. For analysis of variance statistics, the upper summit includes data from 730–770 m and the lower summit, from 770–1,000 m. a, Total megafauna, excluding xenophophores and the solitary coelenterates attached to rock bases on the upper summit. These groups were excluded because they were not reliably visible in *Angus* photographs. There were significant differences between the upper and lower summit ($P=0.036$) and the summit and flank ($P<0.001$). b, Rattail fishes (*N. liolepis*). Fish were ~ 15 –20 cm long. There were significant differences between the upper and lower summit ($P=0.033$) and between the summit and flank ($P<0.001$). c, Total sponges. At least three unidentified species were present. There were significant differences between the summit and flank ($P=0.020$). d, Combined shrimp (*Heterocarpus nesisi* and *Benthescymus altus*) and mysids (*Boreomysis* sp.). Nearly significant differences were observed between summit and flank sites ($P=0.066$). e, Galatheid crabs (*M. c.f. hystrix*). Crabs are ~ 5 -cm long. There were significant differences between upper and lower summit sites ($P=0.003$) and between summit and flank sites ($P<0.001$). f, Unidentified sea pens. These were present only on soft substrates and were ~ 15 -cm long. g, Total macrofauna



retained on a 300- μ m screen. Samples were taken in Ekman-style box cores (196 cm^2 surface area) to a depth of 15 cm. Samples were preserved on board ship in 10% buffered formalin and later transferred to alcohol and sorted in the laboratory. Each data point represents macrofauna from one box core converted to n per m^2 . There were significant differences between upper and lower summit sites ($P=0.003$) and between summit and flank sites ($P=0.051$). h, Total meiofauna (excluding foraminifera) retained on a 63- μ m screen. Samples were taken from one subcore (49 cm^2 surface area) within each box core, processed as described above. Only the upper 5 cm of sediment were sorted for meiofauna. There were significant differences between the upper and lower summit ($P=0.044$). i, Nematode to harpacticoid copepod abundance ratios in the meiofauna cores. There were significant differences between summit and flank sites ($P=0.019$).

aerobic metabolism, although some vertical migrators can transit narrow low oxygen zones, or live anaerobically or at very low metabolic levels (in diapause, for example) for periods of time²³. A pronounced oxygen-minimum zone that is vertically broad is therefore an effective barrier for most plankton. In this situation, the use of particulate material in midwater would be reduced, presumably enhancing benthic particulate fluxes.

Other interactions between the seamount and flow field could also mediate effects of the oxygen minimum. The high regional productivity of the eastern tropical Pacific¹³ could be supplemented near Volcano 7 by topographically induced upwelling²⁴, which could contribute to the particle flux to the benthos. But, no definitive evidence of upwelling into the euphotic zone (which already extended below the mixed layer) was observed.

Current acceleration over topographic features is an alternative process that may enhance local abundances of suspension-feeding megafauna on seamounts²⁵. Although high abundances of some species occurred on ridges, the general megafaunal abundance peak on the summit of Volcano 7 did not correspond to exposed topography and included many non-suspension-feeding taxa.

We hypothesize that the striking benthic zonation on Volcano 7 is due to effects of the oxygen-minimum operating both in the water column (by enhancement of unaltered particulate food supply) and on the benthos (by exclusion of hypoxia-sensitive taxa). Oxygen minima also intersect the sea floor on continental slopes, as in the Arabian Sea⁵, and the distinctive benthic faunal gradients produced by these interactions could be preserved in the palaeoceanographic record^{4,6}. □

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- Longhurst, A. R. *Deep-Sea Res.* **14**, 51–63 (1967).
- Vinogradov, M. E. & Voronina, N. M. *Okeanologiya* **1**, 670–678 (1961). English translation in *Deep-Sea Res.* **9**, 523–530 (1962).
- Sanders, H. L. in *Diversity and Stability in Ecological Systems*, 71–81 (Brookhaven National Laboratory, Upton, New York, 1969).
- Rhoads, D. C. & Morse, J. W. *Limnol. Oceanogr.* **16**, 413–428 (1971).
- Rice, A. L. (ed.). *Deep-Sea Challenge: The John Murray/Mahabiss Expedition to the Indian Ocean, 1933–34* (Unesco, Paris, 1986).
- Mullins, H. T., Thompson, J. B., McDougall, K. & Vercoeur, T. L. *Geology* **13**, 491–494 (1985).
- Batiza, R. *Geology* **8**, 477–482 (1980).
- Wyrtki, K. *Oceanogr. mar. Biol. A Rev.* **4**, 33–68 (1966).
- Knauer, G. A., Martin, J. H. & Bruland, K. W. *Deep-Sea Res.* **26**, 97–108 (1979).
- Gowing, M. M. & Silver, M. W. *Mar. Biol.* **73**, 7–16 (1983).
- DeMaster, D. J., McKee, B. A., Nittroter, C. A., Brewster, D. C. & Biscaye, P. E. *Mar. Geol.* **66**, 133–148 (1985).
- Banck, K. *Limnol. Oceanogr.* **19**, 695–699 (1974).
- Owen, R. W. & Zeitzschel, B. *Mar. Biol.* **7**, 32–36 (1970).
- Lampitt, R. S. *Deep-Sea Res.* **32**, 885–897 (1985).
- Deuser, W. G. *Deep-Sea Res.* **33**, 225–246 (1986).

- Southward, A. J. & Southward, E. C. *ISI Atlas of Science: Animal and Plant Sciences* **1**, 203–207 (1988).
- Levin, L. A. & Smith, C. R. *Deep-Sea Res.* **31**, 1277–1285 (1984).
- Josefson, A. B. & Widom, B. *Mar. Biol.* **100**, 31–40 (1988).
- Raffaelli, D. *Mar. env. Res.* **23**, 135–152 (1987).
- Sewell, R. B. S. & Fage, L. *Nature* **162**, 949–951 (1948).
- Brinton, E. *Prog. Oceanogr.* **8**, 185–189 (1973).
- Sameoto, D. D., Guglielmo, L. & Snelson, M. K. *Mar. Biol.* **96**, 235–245 (1987).
- Childress, J. J. *Comp. Biochem. Physiol.* **50A**, 787–799 (1975).
- Boehlert, G. W. & Genin, A. in *Seamounts, Islands and Atolls* (eds Keating, B. H., Fryer, P., Batiza, R. & Boehlert, G. W.) 319–334 (American Geophysical Union, Washington, DC, 1987).
- Genin, A., Dayton, P. K., Lonsdale, P. F. & Spiess, F. N. *Nature* **322**, 59–61 (1986).
- Carritt, D. E. & Carpenter, J. H. *J. mar. Res.* **24**, 266–318 (1966).
- Wiebe, P. H., Bur, K. H., Boyd, S. H. & Morton, A. W. *J. mar. Res.* **34**, 313–326 (1976).
- Parsons, T. R., Maita, Y. & Lalli, C. M. *A Manual of Chemical and Biological Methods for Seawater Analysis* (Pergamon, Oxford, 1984).
- Hobbie, J. E., Daley, R. J. & Jasper, S. *Appl. env. Microbiol.* **33**, 1225–1228 (1977).

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Evolution of the MHC class I genes of a New World primate from ancestral homologues of human non-classical genes

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THE products of the classical human major histocompatibility complex (MHC) class I genes (*HLA-A*, *-B*, *-C*) are highly polymorphic molecules that bind peptides and present them to T lymphocytes¹⁻⁴. The non-polymorphic, non-classical MHC class I gene products (*HLA-E*, *-F*, *-G*) are not restricting elements for the majority of T lymphocytes⁵⁻⁷. The evolutionary relationship of the non-classical and classical MHC class I genes is unclear. Here we present the cloning and sequencing of the MHC class I genes of a New World primate, the cotton-top tamarin (*Saguinus oedipus*). The expressed MHC class I genes of this species are more closely related to the human non-classical *HLA-G* (ref. 7) gene than they are to genes of the human classical *HLA-A*, *-B*, and *-C* loci. These observations imply that classical and non-classical genes do not necessarily constitute mutually exclusive groups over evolutionary time.

In contrast to other species⁸, the MHC class I diversity of the cotton-top tamarin is limited⁹. Only 11 different MHC class I molecules were found in 79 unrelated tamarins (data not shown). To investigate the reasons for this limited MHC class I diversity, we have cloned full-length MHC class I complementary DNA

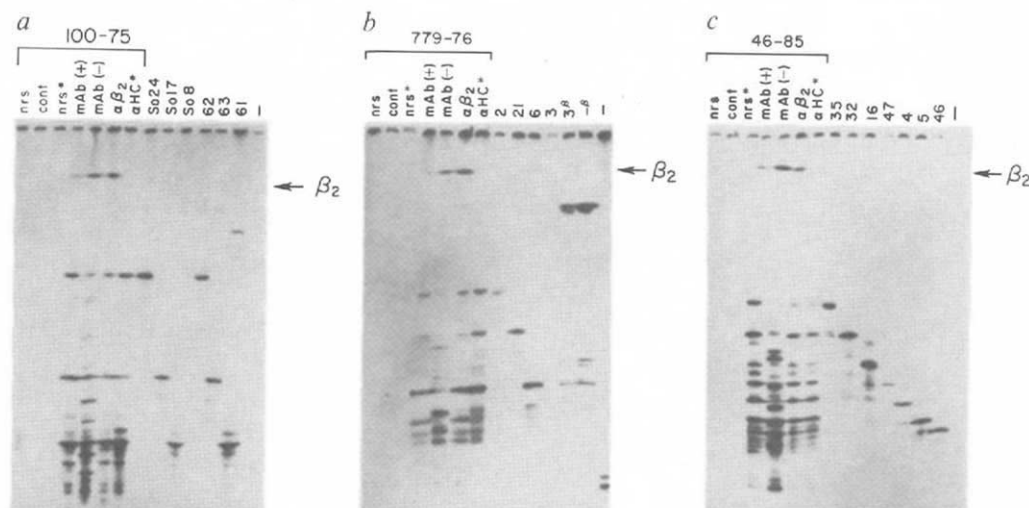
from tamarins using conventional techniques¹⁰ and the polymerase chain reaction¹¹ (PCR). Fortunately, all 11 tamarin MHC class I genes were expressed by the lymphocytes of three animals (numbers 100-75, 779-76 and 46-85). MHC class I cDNAs were isolated from these three animals and transfection of these cloned cDNAs into COS cells demonstrated that we had cloned all of the previously defined expressed tamarin genes (Fig. 1).

To ensure that we had isolated all of the expressed tamarin MHC class I genes, another set of primers was used to amplify exons 2 and 3 from RNA and DNA from animal 100-75. Only the three previously isolated cDNAs were amplified from RNA from this animal. In screening genomic clones, the three previously characterized cDNAs and three additional genes (*N1*, *N2*, *N3*) were defined (data not shown). *N2* was identical to cDNA 3 that had previously been cloned from animal 779-76. It is a gene with a premature termination codon in the cytoplasmic domain that was not expressed on the surface of lymphocytes from either animal 100-75 or 779-76. Both *N1* and *N3* contained deletions that would result in a premature termination codon or disrupt the folding of an MHC class I molecule (data not shown), indicating that they are pseudogenes. Thus, PCR amplification from genomic DNA did not reveal any additional intact tamarin MHC class I genes.

Analysis of the inter-locus variability in the human MHC class I genes shows that genes differ one from another by 50–100 bases in exons 2, 3 and 4. Intra-locus variability ranges from 1–50 bases in the same region¹². Nucleotide sequences of the tamarin MHC class I genes were statistically less variable than those of human and mouse (Table 1). Accordingly, the predicted amino acid sequences of the tamarin MHC class I molecules were quite similar (Fig. 2). This similarity was especially striking in the transmembrane domain, in contrast to the highly variable MHC class I transmembrane domains of other species.

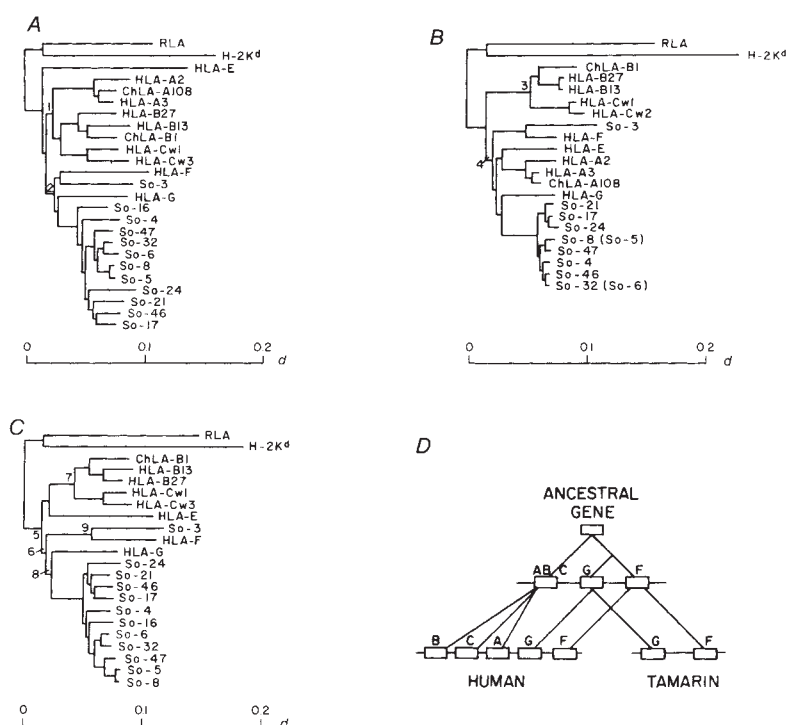
In phylogenetic trees¹³ of exons 1–3, 4–7 and 1–7 (Fig. 3*a–c*), all but one of the tamarin cDNAs clustered with the human non-classical *HLA-G* MHC class I gene. The tamarin cDNA 3 was unique in that it was quite closely related to *HLA-F* (ref. 6). The presence of *HLA-G*- and *HLA-F*-specific substitutions in the predicted amino-acid sequences of the tamarin MHC

FIG. 1 Expression of seventeen cDNAs cloned from the lymphocytes of three cotton-top tamarins. **a**, PCR amplification of cDNA synthesized from RNA was used to clone full-length tamarin MHC class I cDNAs. Three MHC class I heavy chains and β_2 -microglobulin (β_2) were precipitated²⁸ from the lysate of a biosynthetically labelled, EBV-transformed B-cell line from animal 100-75 using the monoclonal antibody BB7.7 (mAb). Precipitates were either left untreated (–) or sialic acid residues were removed with neuraminidase (+). A rabbit antiserum reactive with human β_2 -microglobulin ($\alpha\beta_2$) and one reactive with denatured human heavy chains (α HC) also precipitated the same three tamarin MHC class I molecules. Normal rabbit serum (nrs) and a monoclonal antibody (cont) reactive with an irrelevant determinant were used as controls. Lysates were denatured (*) in some instances. Three cDNAs cloned from a cDNA library made from an EBV-transformed cell line from animal 100-75 (So24, So17 and So8)¹⁰ and three cDNAs amplified by PCR^{11,29} from cDNA synthesized from total cellular RNA isolated from the same cell line (62, 63 and 61) were transfected into COS cells. The sequences of the three PCR-amplified cDNAs (62, 63 and 61) differed by only 1 of 3,000 nucleotides from the sequences of



those that were generated using standard cDNA cloning techniques (So24, So17 and So8). As a control, COS cells were also transfected with the mouse CD20 molecule (–). **b**, COS cells were transfected with four different cDNAs (2, 21, 6 and 3) isolated by PCR amplification of RNA from lectin-stimulated peripheral blood lymphocytes (PBL) from animal 779-76. MHC class I molecules from COS cells transfected with cDNA 3 were also precipitated with an antiserum reactive with human β_2 -microglobulin (β). **c**, COS cells were transfected with seven different cDNAs (35, 32, 16, 47, 4, 5, and 46) isolated by PCR amplification of RNA from lectin-stimulated PBL from animal 46-85.

FIG. 3 The tamarin MHC class I genes are related to the *HLA-G* and *-F* non-classical genes. Trees were constructed for different regions of the MHC class I gene to clarify the relationship between the human and tamarin genes. This was necessary because the *HLA-A* locus, as it now exists, is the product of a recombination between an ancestral *HLA-A* locus and an *HLA-E*-like locus. As a result of this event (which antedated the human-chimpanzee divergence), the sequence of *HLA-A* (and of *ChLA-A*) 3' to the third intron is *HLA-E*-like and not closely related to other human classical MHC class I genes³². Phylogenetic trees based on nucleotide substitutions per site (*d*) among cotton-top tamarin and other primate MHC class I genes: **A**, tree for 591 aligned nucleotides from exons 1–3; **B**, tree for 414 aligned nucleotides from exons 4–7; **C**, tree for 1,005 aligned nucleotides from exons 1–7. In all cases rabbit (*RLA*) and mouse (*H-2K^d*) MHC class I genes are used as outgroups to establish the root of the tree. Significance of selected internal branch lengths was tested by the method of Li³³. In **A**, the internal branch d_{12} (between the human classical gene cluster and the human non-classical/tamarin cluster) is significant at the 5% level ($t=2.05$). In **B**, the internal branch length d_{34} (between the cluster containing tamarin genes, human non-classical genes and this region of the *A* alleles and the cluster containing *B* and *C* alleles) is significant at the 0.1% level ($t=4.10$). In **C**, the internal branch d_{67} (between the cluster containing the tamarin genes, *G* and *F* and the cluster of human and chimpanzee classical genes) is significant at the 0.1% level ($t=4.61$); the internal branch d_{89} (between the *F*/So-3 cluster and the cluster containing *G*, and the other tamarin sequences) is significant at the 0.1% level ($t=6.20$); the internal branch d_{58} (between the *G*-human classical branch point and the *G*-tamarin branch point) is significant at the 5% level ($t=2.09$); and the internal branch length d_{59} (between the *F*-human classical branch point and the *F*-So-3 branch point) is significant at the 0.1% level ($t=6.33$). The fact that d_{58} and d_{59} are significant is evidence that *G*, *F* and the ancestor of human classical



class I genes existed before the separation of Old and New World primates. **D**, Schematic representation of a hypothetical evolutionary history of tamarin and human MHC class I genes. This figure is not intended to represent relative chromosome positions of the various genes.

class I molecules supports the results of this phylogenetic analysis (Fig. 2). Gene trees based on the partial nucleotide sequences available for *N1* and *N3* were also constructed. These two genes were also related to the *HLA-G* locus (data not shown). Thus, all tamarin MHC class I genes have evolved from ancestral homologues of human non-classical MHC class I genes.

We could find no evidence for the presence of tamarin MHC class I gene products related to human *HLA-A*, *-B* or *-C* molecules. Antibodies against human MHC class I molecules precipitated only the three *HLA-G*-related products from a B-cell line from animal 100-75. Synthesis of a cDNA library from this cell line using conventional techniques¹⁰ and PCR amplification using two different primer pairs also demonstrated that only these three MHC class I genes were expressed. As tamarins can mount CD8⁺ cytotoxic T-cell responses¹⁴, it is likely that their *HLA-G*-related MHC class I molecules are functional. These observations suggest that *HLA-G*-related MHC class I genes constitute the classical MHC class I genes

of the tamarin. This implies that classical and non-classical MHC class I genes need not constitute mutually exclusive groups over evolutionary time.

A phylogenetic analysis of human and tamarin MHC class I sequence data (Fig. 3) suggests that the last common ancestor of humans and tamarins had at least the following MHC class I genes: *G*, *F* and the ancestors of *A*, *B* and *C*. Both the tamarin MHC class I *G*-related and *F*-related genes appear to have diverged from the ancestor of the *HLA-A*, *-B* and *-C* genes. This hypothesis is supported by the significant branch length (d_{58} , Fig. 3c) between *G* and the branch point of the ancestor of *HLA-A*, *-B* and *-C* (5) and *G* and the branch point of the tamarin *G*-related genes (8). The *F* gene also diverged from *G* after *G* had split from the ancestor of *A*, *B* and *C* as indicated by a similar significant internal branch length (d_{59} , Fig. 3c). Therefore, as both *G* and *F* diverged from *ABC* before the derivation of the tamarin genes and as *G* and *F* are both present in tamarins and humans, the MHC chromosome of the last common ancestor of humans and tamarins probably had genes for *G*, *F* and *ABC* (Fig. 3d).

Hybridization studies¹⁵ and sequence analyses¹⁶ had suggested that non-classical MHC class I genes may not be conserved in different orders of mammals. The isolation of a chimpanzee *HLA-F* cDNA¹², however, demonstrates that non-classical loci can be shared by closely related mammalian species. The finding in the tamarin of a gene related to *HLA-F* that is not expressed shows that non-classical MHC class I loci can be preserved for much longer than was previously thought.

The remarkable lack of variation and polymorphism at the MHC class I loci of the tamarin may explain, in part, the extreme susceptibility of these New World primates to a variety of viral pathogens. Tamarins succumb to a fatal lymphoproliferative syndrome when infected with Epstein-Barr virus (EBV), *Herpesvirus saimiri* and *H. ateles*^{17,18}. They also die following infection with Rous sarcoma virus, measles and parainfluenza viruses¹⁷.

TABLE 1 The variability of the expressed tamarin MHC class I genes is significantly lower than the variability of the human and mouse MHC class I genes

Species	Comparison			
	no.	$d \pm \text{s.e.m.}$	$d_s \pm \text{s.e.m.}$	$d_N \pm \text{s.e.m.}$
Cotton-top tamarin	55	4.7 ± 0.4	4.8 ± 0.8	4.7 ± 0.5
Human	66	$8.7 \pm 0.6^*$	$13.9 \pm 1.7^*$	$7.1 \pm 0.6^*$
Mouse	36	$8.2 \pm 0.5^*$	7.1 ± 1.0	$8.6 \pm 0.6^*$

Comparisons of mean numbers of nucleotide substitutions per site (d), per synonymous site (d_s) and per non-synonymous site (d_N); expressed as percentages in alpha-1, alpha-2 and alpha-3 domains (822 nucleotides) of cotton-top tamarin, human and mouse MHC class I molecules. d is estimated by the Jukes-Cantor method²⁴, d_s and d_N are estimated by the Nei and Gojobori method²⁵, standard errors are estimated by the Nei and Jin method²⁶. See ref. 27 for human and mouse sequence references.

* Means for mouse and human that differ significantly (0.1% level) from the corresponding means for tamarin.

As individual MHA class I glycoproteins can probably bind and present only a restricted number of viral peptides to T cells, reduced variability in the species' MHC class I glycoproteins may render tamarin MHC class I molecules unable to bind a diversity of viral peptides. This may leave the tamarin susceptible to fatal infections by pathogens to which it is not exposed in its natural environment.

Finally, it is interesting to speculate as to the evolutionary pressures that might have selected for deletion of the *HLA-A*, *-B*, and *-C* in the tamarin and utilization of *HLA-G*-related MHC class I genes. Most tamarins are born as stable bone

marrow-chimaeric fraternal twins¹⁹. MHC class I similarity between genetically disparate, coexisting cell populations would facilitate this chimaerism. Indeed, it has recently been demonstrated that cells at the interface of the human maternal and fetal circulations in the extravillous trophoblast do not express detectable classical HLA class I molecules^{20,21}. Rather, these fetal cells express products of the non-polymorphic, non-classical *HLA-G* genes^{22,23}. Utilization of relatively non-polymorphic MHC class I genes may be a mechanism by which MHC class I functions can be served with a minimum stimulation of alloreactivity. □

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1. Parham, P. *et al. Proc. natn. Acad. Sci. U.S.A.* **85**, 4005-4009 (1988).
2. Townsend, A. R. M. *et al. Cell* **44**, 959-968 (1986).
3. Bjorkman, P. J. *et al. Nature* **329**, 506-512 (1987).
4. Bjorkman, P. J. *et al. Nature* **329**, 512-518 (1987).
5. Koller, B. H., Geraghty, D. E., Shimizu, Y., DeMars, R. & Orr, H. T. *J. Immun.* **141**, 897-904 (1988).
6. Geraghty, D. E., Wei, X., Orr, H. T. & Koller, B. H. *J. exp. Med.* **171**, 1-18 (1990).
7. Geraghty, D. E., Koller, B. H. & Orr, H. T. *Proc. natn. Acad. Sci. U.S.A.* **84**, 9145-9149 (1987).
8. Klein, J. *Natural History of the Major Histocompatibility Complex* (Wiley, New York, 1986).
9. Watkins, D. I., Hodi, F. S. & Letvin, N. L. *Proc. natn. Acad. Sci. U.S.A.* **85**, 7714-7718 (1988).
10. Watkins, D. I., Letvin, N. L., Hughes, A. L. & Tedder, T. F. *J. Immun.* **144**, 1136-1143 (1990).
11. Saiki, R. K. *et al. Science* **230**, 1350-1354 (1985).
12. Lawlor, D. A., Ward, F. E., Ennis, P. D., Jackson, A. P. & Parham, P. *Nature* **335**, 268-271 (1988).
13. Saitou, N. & Nei, M. *Molec. biol. Evol.* **4**, 406-425 (1987).
14. Picus, J., Aldrich, W. R. & Letvin, N. L. *Transplantation* **39**, 297-303 (1985).
15. Rogers, J. H. *EMBO J.* **4**, 749-753 (1985).
16. Hughes, A. L. & Nei, M. *Molec. biol. Evol.* **6**, 559-579 (1989).
17. Wolfe, L. G. & Deinhardt, F. *Primates Med.* **10**, 96-118 (1978).
18. Miller, G. *et al. J. exp. Med.* **145**, 948-967 (1977).
19. Benirschke, K., Anderson, J. M. & Brownhill, L. E. *Science* **138**, 513-515 (1962).

20. Ellis, S. A., Sargent, I. L., Redman, C. W. G. & McMichael, A. J. *Immunology* **59**, 595-601 (1986).
21. Stern, P. L. *et al. Hum. Immun.* **22**, 247-261 (1988).
22. Ellis, S. A., Palmer, M. S. & McMichael, A. J. *J. Immun.* **144**, 731-735 (1990).
23. Kovats, S. *et al. Science* **248**, 220-223 (1990).
24. Jukes, T. H. & Cantor, C. R. *Mammalian Protein Metabolism* (ed. Munro, H. N.), 21-132 (Academic, New York, 1969).
25. Nei, M. & Gojobori, T. *Molec. biol. Evol.* **3**, 418-426 (1986).
26. Nei, M. & Jin, L. *Molec. biol. Evol.* **6**, 290-301 (1989).
27. Hughes, A. L. & Nei, M. *Nature* **335**, 167-170 (1988).
28. Watkins, D. I., Kannagi, M., Stone, M. E. & Letvin, N. L. *Eur. J. Immun.* **18**, 1425-1432 (1988).
29. Newman, P. J. *et al. J. clin. Invest.* **82**, 739-743 (1988).
30. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463-5467 (1977).
31. Ennis, P. D., Zemmour, J., Salter, R. D. & Parham, P. *Proc. natn. Acad. Sci. U.S.A.* (in the press) (1990).
32. Hughes, A. L. & Nei, M. *Genetics* **122**, 681-686 (1989).
33. Li, W.-H. *Molec. biol. Evol.* **6**, 424-435 (1989).

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Inhibition by brefeldin A of presentation of exogenous protein antigens to MHC class II-restricted T cells

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PEPTIDES bound to class I or class II major histocompatibility complex (MHC)-encoded molecules are ligands for the antigen-specific T-cell receptor of T-cells carrying the CD8 and CD4 antigens, respectively¹. MHC class I-restricted T cells generally recognize peptides derived from processing of endogenously synthesized cellular antigens, whereas class II-restricted T cells usually recognize peptides derived from exogenous antigens entering antigen presenting cells. Accordingly, two separate pathways of antigen processing and presentation have been proposed^{2,3}. The fungal metabolite brefeldin A (BFA)⁴, an inhibitor of protein transport from the endoplasmic reticulum⁵⁻⁷, inhibits presentation of endogenous antigens for MHC-restricted T-cell recognition⁸. The selectivity of BFA activity has been inferred to reflect presentation of a given antigen processed through the cytosolic or the endocytic route^{9,10}. Here we show that BFA also greatly inhibits the presentation of exogenous protein antigens by MHC class II molecules to T cells, indicating a broader effect of this drug on antigen presentation and an additional similarity between the two processing pathways. As cycloheximide, a protein synthesis inhibitor, also inhibits presentation of protein antigens to class II-restricted T cells, the data indicate that peptides generated by processing of exogenous proteins bind to newly synthesized class II molecules for presentation to T cells.

To test the effect of BFA on the presentation of exogenous protein antigens to MHC class II-restricted T cells, LK-35.2

cells (a B-cell hybridoma expressing I-A^{k,d} and I-E^{k,d} molecules) were treated with BFA during a 4-h pulse with hen egg-white lysozyme (HEL) or chicken ovalbumin (Ova). After washing these antigen presenting cells (APC), antigen presentation to the HEL-specific T-cell hybridoma 1H11.3 or Ova-specific hybridoma DO11.10 was assessed by measuring T-cell growth factor (interleukins 2 and 4) production. Incubation of APC with 3 µM BFA abrogates their capacity to present exogenous protein antigens (Fig. 1a,b). If, under the same conditions, APC are pulsed with the HEL peptide 105-120 (recognized by hybridoma 1H11.3 with I-E^d molecules¹¹) or with the Ova peptide 323-339 (recognized by hybridoma DO11.10 with I-A^d molecules¹²), BFA has no effect. Titration of interleukins produced by peptide-activated T-cell hybridomas also did not show any effect of BFA on antigen presentation (data not shown). These results demonstrate that BFA inhibits the presentation of exogenous soluble proteins recognized by T cells with MHC class II molecules, but has no effect on the ability of APC to present synthetic peptides.

The effect of BFA on antigen presentation can be partially overcome by increasing the concentration of protein antigen during pulsing, using T-cell hybridomas responding to low antigen concentration (Fig. 1c-h). A similar effect is also exerted on LK-35.2 cells by cycloheximide, although this was not entirely predicted by previous work^{13,14}. In fact, a remarkable concordance between BFA and cycloheximide in the inhibition of exogenous antigen presentation is observed. These results suggest that a less efficient, secondary pathway for class II-restricted antigen presentation, independent of *de novo* protein synthesis or of protein egress from the endoplasmic reticulum (ER), may be activated by pulsing the APC with high (at least 20-fold molar excess) protein antigen concentrations. Alternatively, the data may reflect incomplete inhibition of protein egress from the ER. Conversely, the lysosomotropic agent chloroquine inhibits antigen presentation at any concentration of protein antigen tested, indicating that BFA does not act as a lysosomotropic agent.

The inhibitory effect of BFA on the presentation of soluble proteins to class II-restricted T cells can be demonstrated in different types of APC, including B-hybridoma and B-lymphoma

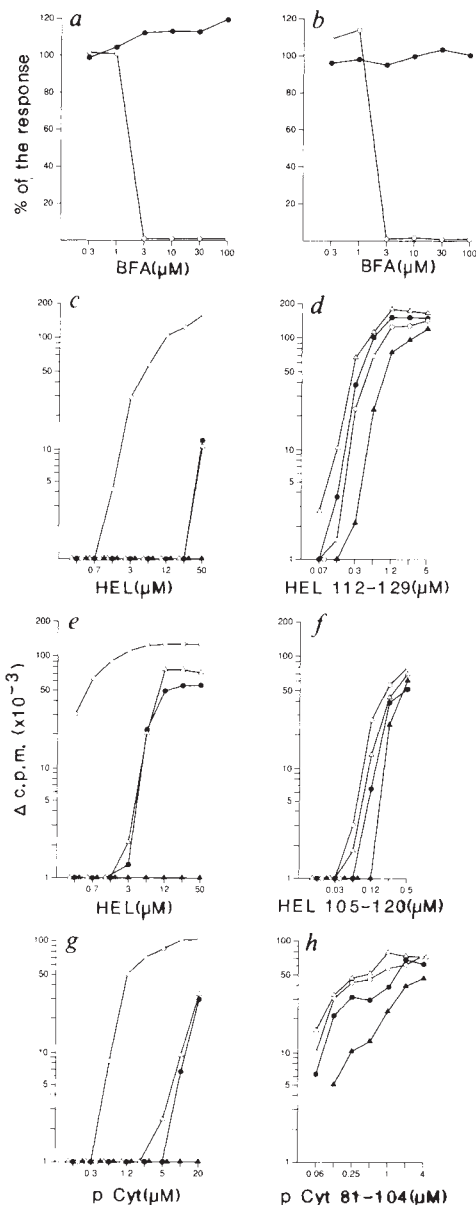


FIG. 1 Inhibition of antigen presentation by BFA. LK-35.2 APC (2.5×10^4 per well) were incubated with the indicated concentrations of BFA (Sandoz; M_r 280) and 15 min later antigen was added: *a*, 2 μ M HEL ($\circ$) or 2 μ M HEL peptide 105–120 ($\bullet$); *b*, 250 μ M Ova ($\circ$) or 1 μ M Ova peptide 323–339 ($\bullet$). After 4-h incubation at 37 $^{\circ}$ C, the APC were washed three times and 5×10^4 cells per well of hybridoma 1H11.3 (*a*) or D011.10 (*b*) were added. After 24 h, antigen-specific T-cell growth factor production was determined by 3 H-labelled thymidine incorporation into CTLL cells (ref. 21). Data are presented as percentage of the c.p.m. (arithmetic mean of triplicate cultures) obtained in response to the protein antigen or to the peptide. Control responses in the absence and presence of protein and peptide were respectively: 660/75,578 and 515/81,181 (*a*); 528/54,904 and 568/93,175 (*b*). *c–h* LK-35.2 cells were incubated with 20 μ M BFA (Δ), 350 μ M cycloheximide ($\bullet$) (Sigma), 200 μ M chloroquine ($\blacktriangle$) (Sigma), or left untreated ($\circ$). These compounds were added to APC 15 min, 2 h or 30 min, respectively, before addition of the indicated concentrations of protein antigens or synthetic peptides. After 4-h incubation with antigen, the APC were washed, fixed with 0.05% glutaraldehyde²¹, washed again and the HEL-specific T-cell hybridomas 2C8.4, recognizing the HEL peptide 112–129 in association with I-A^k molecules²¹ (*c, d*), 1H11.3 (*e, f*), or the pigeon cytochrome c (p Cyt)-specific T-cell hybridoma 2B4.11, recognizing the p Cyt peptide 81–104 in association with I-E^k molecules²² (*g, h*), were added. The experiment was continued as in *a*, results are expressed as c.p.m. of 3 H-labelled thymidine incorporation into CTLL cells. Background proliferation of CTLL cells was 201 (*c, d*), 136 (*e, f*) and 150 (*g, h*) c.p.m.

METHODS. The synthesis, purification and analysis of HEL peptides and the establishment of T-cell hybridomas have been described previously^{11,21}. Cultures containing 5×10^4 T-hybridoma cells and 2.5×10^4 LK-35.2 cells (American Type Culture Collection) were set up with or without antigen in 0.2 ml RPMI 1640 medium (Gibco) supplemented with 2 mM L-glutamine, 50 μ M 2-mercaptoethanol, 50 μ g ml⁻¹ gentamicin and 10% FCS (Seromed). After 24 h of culture, 50 μ l aliquots of supernatant were assayed for the presence of T-cell growth factors by 3 H-labelled thymidine incorporation in 10^4 CTLL cells¹¹.

cells (Fig. 2*a*), resident peritoneal adherent cells (Fig. 2*b*) and MHC class II-transfected L-cell fibroblasts (Fig. 2*c*). In the latter case, fibroblasts transfected only with I-E^d genes¹⁵ or supertransfected with the invariant chain gene¹⁶, were equally sensitive to BFA, suggesting that expression of the invariant chain does not influence the inhibition by BFA of protein antigen presentation to class II-restricted T-cells.

Addition of BFA to APC 1 h after HEL induces a much reduced effect on antigen presentation, and no effect 2 h after HEL, indicating that during this period a sufficient amount of protein had been processed to yield antigenic complexes stimulating an optimal T-cell response (Fig. 3*a*). Addition of BFA to APC any time after the HEL peptide 105–120, is ineffective (Fig. 3*b*). The inhibition of class II-restricted antigen presentation by BFA is rapidly and completely reversible, and washing the APC after 4-h incubation with BFA, just before the addition of HEL

FIG. 2 Inhibition by BFA of the presentation of HEL by different APC. APC were incubated with the indicated concentrations of BFA and 15 min later 2 μ M HEL (open symbols) or 2 μ M HEL peptide 105–120 (closed symbols) were added. *a*, LK-35.2 (circles), A20 cells (triangles). *b*, Resident peritoneal cells enriched in macrophages by plastic adherence from BALB/c (circles) or DBA/2 mice (triangles). *c*, CA36.2.1 fibroblasts transfected with I-E^d genes¹⁵ (circles) or CA36.2.1.li supertransfected with the invariant chain gene¹⁶ (triangles). The experiment was then continued as in Fig. 1. Control responses of the I-E^d-restricted T-cell hybridoma 1H11.3 in the absence and presence of HEL and the HEL peptide 105–120 were respectively: 1019/134,776 and 861/142,914 (LK-35.2); 816/25,207 and 855/78,439 (A20); 711/45,344 and 435/31,413 (BALB/c); 863/61,174 and 671/46,062 (DBA/2); 285/36,898 and 346/95,105 (CA36.1.2); 360/21,515 and 256/76,398 (CA36.1.2.li).

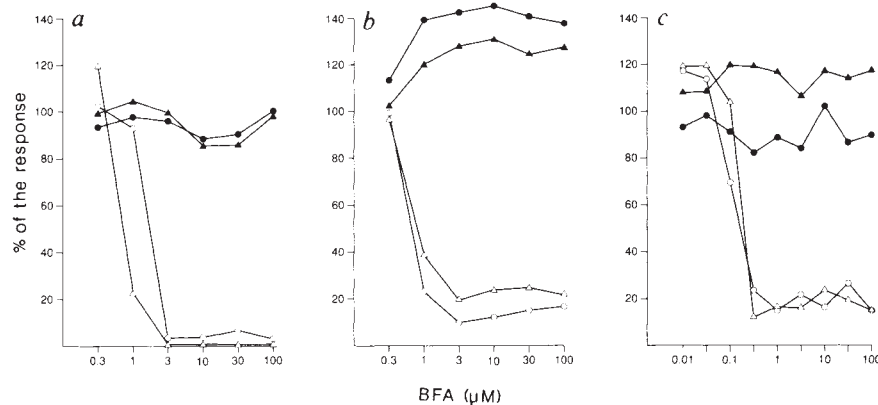
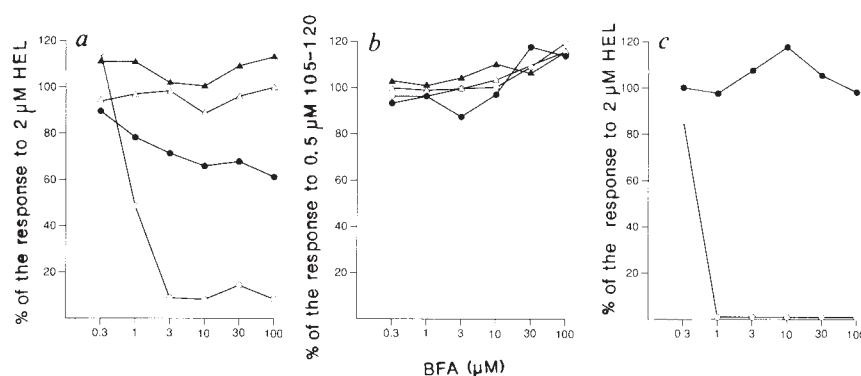


FIG. 3 The inhibition by BFA of HEL presentation is reversible and requires the presence of the drug during the antigen processing phase. *a, b*, LK-35.2 cells were incubated with 2 μ M HEL (*a*), or 0.5 μ M HEL peptide 105–120 (*b*) and the indicated concentrations of BFA were added 15 min before antigen (○), or 1 h (●), 2 h (△), 3 h (▲) after antigen. The experiment was then continued as in Fig. 1. Control responses of hybridoma 1H11.3 in the presence or absence of HEL and HEL peptide 105–120 were respectively: 745/164,340 and 816/165,197 c.p.m. *c*, LK-35.2 cells were incubated with the indicated concentrations of BFA and 15 min later 2 μ M HEL was added. After 4 h, APC were washed, the I-E^d-restricted HEL-specific T-cell hybridoma 1E5.11 (ref. 11) was added, and the experiment continued as in Fig. 1 (○). Alternatively, LK-35.2 cells were incubated with BFA for 4 h, washed, pulsed with HEL for 4 h and the



experiment continued as above (●). Control responses in the absence or presence of HEL were respectively 883 and 27,391 c.p.m.

and T-hybridoma cells, restores interleukin-2 (IL-2) production to control levels (Fig. 3c).

The effect of BFA on antigen presentation does not seem to be related to the amount of surface class II molecules expressed. No difference could be detected by surface immunofluorescence with anti-I-E^d antibody between untreated and BFA-treated cells (Fig. 4c), or in their antigen-presenting function, as peptide presentation to class II-restricted T cells is not affected by BFA treatment of APC.

But BFA affects the biosynthetic maturation of MHC class II molecules, as demonstrated by pulse-chase labelled LK-35.2 cells. Cells were immunoprecipitated with anti-I-A and anti-I-E monoclonal antibodies immediately after labelling and after a 3.5-h chase period. BFA retarded the biosynthetic maturation of both I-A (Fig. 4a) and I-E (data not shown) class II molecules, as lower relative molecular mass (M_r) α - and β -chain species were observed in BFA-treated (Fig. 4a; lanes 3, 4) but not in untreated cells (Fig. 4a; lanes 1, 2). These lower M_r species were sensitive to endoglycosidase H (endo H) treatment

before and at the end of the 3.5-h chase period (Fig. 4b; lanes 6, 8), whereas class II molecules from untreated cells were sensitive to endo H treatment before the chase period, but acquired endo H resistance during 3.5-h post synthesis (Fig. 4b; lanes 2 and 4, respectively). The lower M_r I-A^k species present in BFA-treated cells before the chase period (Fig. 4a, lane 3 and b, lane 5) was converted to slightly larger species during the 3.5-h chase period (Fig. 4a, lane 4 and b, lane 7). Furthermore, an increased ability of the monoclonal antibody to immunoprecipitate I-A^k molecules after the chase period in BFA-treated cells is apparent (compare Fig. 4a, lanes 3, 4, and b, lanes 5, 7). This may represent continued processing of some of the carbohydrate chains of the I-A^k molecule during the BFA treatment. A similar situation has been described for the T-cell receptor α - and γ -chains, and was accounted for by the activity of *cis*-medial Golgi glycosidases and glycosyltransferases redistributed by BFA in the ER⁶. In contrast to the T-cell receptor α -chain, I-A^k molecules retain some sensitivity to endo H (Fig. 4b, lane 8). Thus, BFA treatment prevents complete maturation

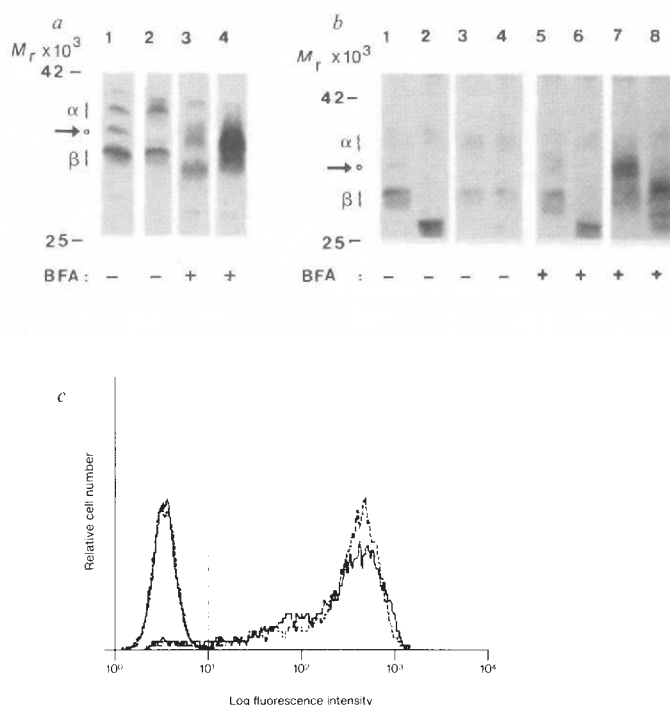


FIG. 4 Effect of BFA on the expression of class II MHC molecules. *a*, Anti-I-A^k immunoprecipitates from BFA-untreated (lanes 1, 2) or -treated (lanes 3, 4) cell lysates at 0 h (lanes 1, 3) or 3.5 h (lanes 2, 4) after start of chase period. *b*, Endo H sensitivity of class II molecules. Anti-I-A^k immunoprecipitates of lysates from 0 h (lanes 1, 2, 5, 6) and 3.5 h (lanes 3, 4, 7, 8) after the start of the chase period were digested with endo H. Lanes 1–4 and 5–8 represent immunoprecipitates from control and BFA-treated cell lysates, respectively. Mock- and endo H-digested immunoprecipitates are lanes 1, 3, 5, 7 and 2, 4, 6, 8, respectively. The upper and lower brackets to the left correspond to the position of the α - and β -chains of class II molecules, respectively, and the arrow to the position of the class II-associated invariant chain. The position of M_r standards is indicated. *c*, Cell surface expression of class II molecules. LK-35.2 cells were left untreated (solid line) or incubated with 100 μ M BFA (broken line) for 4 h before staining with anti-I-E monoclonal antibody.

METHODS. *a, b*, LK-35.2 cells ($2.5 \times 10^6 \text{ ml}^{-1}$) were incubated with 10 μ M BFA or left untreated and 15 min later metabolically labelled with 250 $\mu\text{Ci ml}^{-1}$ [³⁵S]methionine (Dupont, 1,100 Ci mmol^{-1}) in methionine-free medium containing 2.5% dialysed FCS for 30 min. Cells were then immediately harvested or chased for 3.5 h in complete medium, lysates were prepared from 5×10^6 cells per time point and immunoprecipitated as described²³ with the anti-I-A^k monoclonal antibody 10.2.16 or with an irrelevant antibody (not shown) and the resulting immune complexes washed as described²⁴. Immunoprecipitates were analysed on 12% (*a*) or 10% (*b*) SDS-PAGE²⁵. Immunoprecipitates were dissociated by heating for 5 min at 90 °C in 40 μl of 0.5% SDS. Half of the solubilized preparation was digested at 30 °C with 8 mU endo H (Genzyme) in 50 mM sodium phosphate buffer, pH 6.5, 0.05% SDS (lanes 2, 4) and the other half mock-digested (lanes 1, 3). After 18 h, 10 μg RNA carrier transfer was added, the digest precipitated by adding four volumes of cold acetone, the precipitate centrifuged at 10,000g (10 min), and the resulting pellet analysed by SDS-PAGE. *c*, Cells were washed, incubated with biotinylated monoclonal antibody 14-4-4 (anti-I-E^d), washed, incubated with fluorescein isothiocyanate (FITC)-streptavidin and analysed using FACStar (Becton-Dickinson). The left peaks represent the fluorescence of cells not incubated with antibody.

of class II molecules in LK-35.2 cells, consistent with the BFA-induced inhibition of glycoprotein carbohydrate maturation due to failure of nascent proteins to leave the ER^{5,6}.

Our data demonstrate that the presentation of soluble exogenous proteins by class II molecules is, in contrast to earlier work¹⁰, BFA-sensitive. The reason for this difference is not clear but could reflect the type of antigen, particulate or soluble, used. It has been suggested that BFA may not inhibit pigeon cytochrome *c* presentation by B-cell hybridomas to class II-restricted T-cells⁸. But the presentation of pigeon cytochrome *c* by LK-35.2 cells to the T-cell hybridoma 2B4.11 is clearly inhibited by BFA (Fig. 1g, h). It has also been suggested that BFA inhibits antigen presentation by macrophages but not by B-cell lymphomas¹⁷. In contrast, our data demonstrate a similar sensitivity to BFA for all the APC tested.

Different explanations could be envisaged for the BFA inhibition of exogenous antigen presentation to MHC class II-restricted T-cells: prevention of peptide generation, of peptide-class II association, or of egress of newly synthesized class II molecules from the ER. The first possibility is unlikely as BFA is probably not a lysosomotropic agent^{5,8}, and does not inhibit aspartyl, serine or metalloproteases (H. Wagner, unpublished data). But BFA might inhibit processing of exogenous antigens if processing requires the transport of newly synthesized proteolytic enzymes into the endosomes. The second possibility is not true for synthetic peptides, which can also directly bind to surface class II molecules. Although BFA may inhibit the association of intracellular peptides to class II molecules, peptides can still bind to MHC class I molecules in BFA-treated cells¹⁸. As BFA blocks protein egress from the ER, the third possibility would predict that encounter, likely in a *trans*- or post-Golgi location, of newly synthesized class II molecules with peptides derived by processing of exogenous proteins, could be prevented by BFA treatment of APC. Cycloheximide also inhibits antigen presentation, indicating that a continuous flow of newly synthesized MHC class II molecules from the ER is required for efficient presentation to T-cells of peptides generated by processing of exogenous proteins. This requirement does not contradict the hypothesis that MHC class II/peptide complexes, after being expressed on the cell surface, may recycle to endocellular organelles allowing peptide exchange to take place^{19,20}. □

Tyrosine phosphatase CD45 is essential for coupling T-cell antigen receptor to the phosphatidyl inositol pathway

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STIMULATION of T lymphocytes through their antigen receptor (T-cell receptor; TCR) results in the activation of a tyrosine kinase^{1,2} and the generation of phosphatidyl inositol (PtdIns)-derived second messengers³⁻⁵. Several reports have indicated that CD45, a haematopoietic cell-specific surface glycoprotein with tyrosine phosphatase activity in its cytoplasmic domain⁶⁻¹⁰, is important in lymphocyte activation¹¹⁻¹⁴. To examine the possibility that CD45 might influence proximal signal transduction events through the TCR, we have isolated a variant of the human T-cell leukaemic line, HPB-ALL, which fails to express this phosphatase. Unlike cells expressing CD45, stimulation of the TCR in the CD45-negative cell does not result in PtdIns-derived second messengers. Reconstitution of CD45 expression restored early signalling events through the TCR. To localize the site of CD45 action, the human muscarinic type 1 receptor, which also activates the PtdIns second messenger pathway^{15,16}, was transfected into the CD45-negative cell. Although stimulation of the TCR failed to generate PtdIns-derived second messengers, there was normal activity of the PtdIns pathway when human muscarinic receptor type 1 was stimulated, despite the absence of CD45. These data indicate that CD45 influences a cellular component that is essential for effective coupling of the TCR to the PtdIns second messenger pathway.

We screened a number of human T-cell leukaemic lines for surface expression of CD45. One of the lines screened, HPB-ALL, stained heterogeneously with a panel of monoclonal antibodies (mAb) directed against CD45. Subcloning resulted in the isolation of several CD45-negative and CD45-positive HPB-ALL variants. Figure 1a-d shows flow cytometry histograms of representative isolates of these cells stained for surface expression of the TCR complex and CD45. In contrast to a previously described mutant cell line deficient in CD45 surface expression¹¹, northern blot analysis revealed no messenger RNA for CD45 in our negative variant, HPB.45.0 (data not shown). HPB.45.0 expresses significantly more TCR than does the CD45-positive variant, HPB.45.1 as assessed with anti-CD3 mAb.

We next examined the possible role of the CD45 tyrosine phosphatase in early events associated with TCR-mediated signal transduction. The ability of anti-CD3 mAb to activate the PtdIns signal transduction pathway was evaluated by measuring changes in cytoplasmic free Ca²⁺ ([Ca²⁺]_i) (Fig. 2). In HPB.45.1, the cell expressing CD45, a rapid and large increase in [Ca²⁺]_i was seen in response to the activating anti-CD3 mAb, Leu 4 (Fig. 2b), similar to increases in [Ca²⁺]_i previously reported for HPB-ALL and other T cells^{3,17,18}. By contrast, no detectable increase in [Ca²⁺]_i was observed in the CD45-negative cell HPB.45.0 (Fig. 2a), even though this cell expresses over an order of magnitude more TCR than the CD45-positive cell. That the cells were adequately loaded with the calcium-sensitive fluor,

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- Möller, G. (ed.) *Immun. Rev.* **106**, 1-187 (1988).
- Morrison, L. A., Lukacher, A. E., Braciale, V. L., Fan, D. & Braciale, T. J. *J. exp. Med.* **163**, 903-914 (1986).
- Germain, R. N. *Nature* **332**, 687-690 (1986).
- Harri, E., Loeffler, W., Sigg, H. P., Stahelin, H. & Tamm, H. *Helv. chim. Acta* **46**, 1235-1243 (1963).
- Fujiwara, T., Oda, K., Yokota, S., Takatsuki, A. & Ikehara, Y. *J. biol. Chem.* **263**, 18545-18552 (1988).
- Lippincott-Schwartz, J., Yuan, L. C., Bonifacio, J. S. & Klausner, R. D. *Cell* **56**, 801-813 (1989).
- Takatsuki, A. & Tamura, G. *Agric. Biol. Chem.* **49**, 899-902 (1985).
- Nuchtern, J. G., Bonifacio, J. S., Biddison, W. E. & Klausner, R. D. *Nature* **339**, 223-226 (1989).
- Yewdell, J. W. & Bennink, J. R. *Science* **244**, 1072-1075 (1989).
- Nuchtern, J. G., Biddison, W. E. & Klausner, R. D. *Nature* **343**, 74-76 (1990).
- Adorini, L. et al. *Proc. natn. Acad. Sci. U.S.A.* **85**, 5181-5185 (1988).
- Shimonevitz, R., Kappler, J., Marrack, P. & Grey, H. *J. exp. Med.* **158**, 303-316 (1983).
- Jensen, P. E. *J. Immun.* **141**, 2545-2550 (1988).
- Harding, C. V. & Unanue, E. R. *J. Immun.* **142**, 12-19 (1989).
- Shastri, N., Malissen, B. & Hood, L. *Proc. natn. Acad. Sci. U.S.A.* **82**, 5885-5889 (1985).
- Stockinger, B. et al. *Cell* **56**, 683-689 (1989).
- Unanue, E. R., Harding, C. V., Luescher, E. F. & Roof, R. W. *Progress in Immunology VII* (eds Melchers, F. et al.) 52-59 (Springer, Berlin, 1989).
- Townsend, A. et al. *Nature* **340**, 443-448 (1989).
- Adorini, L., Appella, E., Doria, G., Cardinaux, F. & Nagy, Z. A. *Nature* **342**, 800-803 (1989).
- Cresswell, P. *Nature* **343**, 593-594 (1990).
- Adorini, L., Appella, E., Doria, G. & Nagy, Z. A. *J. exp. Med.* **168**, 2091-2104 (1988).
- Hedrick, S. M. et al. *Cell* **30**, 141-152 (1982).
- Ullrich, S. J., Robinson, E. A. & Appella, E. *Molec. Immun.* **23**, 545-555 (1986).
- Simonis, S., Miller, J. & Cullen, S. E. *J. Immun.* **143**, 3619-3625 (1989).
- Laemmli, U. K. *Nature* **227**, 680-685 (1970).

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Indo-1, was shown by the response to the calcium ionophore, ionomycin.

The increase in $[Ca^{2+}]_i$ seen after stimulation of the TCR is due to the generation of soluble inositol phosphates by the hydrolysis of phosphatidyl inositol 4,5-bisphosphate (PtdIns(4,5) P_2) (refs 4, 19). We therefore studied the ability of anti-CD3 mAb to induce PtdIns(4,5) P_2 breakdown in the CD45-negative and -positive cells. Figure 3 depicts the result of such an experiment where inositol phosphates were measured at various times after anti-CD3 stimulation of HPB.45.0 and HPB.45.1. As expected from the observed increases in $[Ca^{2+}]_i$, the CD45-positive cells consistently generated an increase in soluble inositol phosphates, whereas the CD45-negative cells showed no such response.

Because activating stimuli alter expression of CD45 in T cells²⁰, we tested the effect of phorbol myristyl-13-acetate (PMA) treatment on CD45 levels in the CD45-negative cells. Following 24 h of PMA incubation, there was low but detectable induction of expression of CD45, peaking at ~48 h (Fig. 1e, f), and correlating with induction of CD45 mRNA (data not shown). To our knowledge, this is the first example of induction of the CD45 gene due to an external stimulus. The PMA-treated cells were stimulated with anti-CD3 mAb and analysed for increases in $[Ca^{2+}]_i$. Figure 2c shows a rapid and sustained increase in $[Ca^{2+}]_i$ seen in response to the Leu 4 mAb in PMA-treated HPB.45.0 cells. As expected, analysis of soluble inositol phosphates after anti-CD3 stimulation revealed substantial increases in the PMA-treated cells (data not shown). Similar studies with the CD45-positive HPB.45.1 cells revealed that PMA induced an increase in CD45 expression and a corresponding increase in PtdIns turnover (data not shown).

To demonstrate more directly that the defect in PtdIns response in the HPB.45.0 is due to its failure to express CD45,

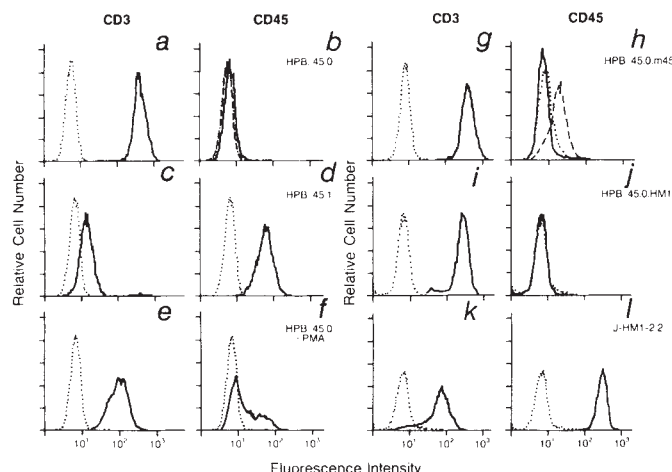


FIG. 1 Surface expression of TCR, human CD45 and murine CD45 on various human T cells. HPB.45.0 (a, b) and HPB.45.0.m45 (g, h) were stained with a control mouse IgG (dotted line); anti-CD3 (Leu4, solid line); anti-human CD45 (HLE, directed against a common determinant of all isoforms of human CD45, solid line); and anti-murine CD45 (M1/9.3.4.HL.2, dashed line). HPB.45.1 (c, d); HPB.45.0 treated with PMA (Sigma), 50 ng ml⁻¹ (48 h; e, f); HPB.45.0.HM1 (i, j), and J-HM1-2.2 (k, l) were stained with control IgG, anti-CD3, and anti-human CD45.

METHODS. Cells were stained as previously described²⁶. Antibodies were from Becton-Dickinson except for M1/9.3.4.HL.2 which was from the ATCC, and FITC-conjugated second antibody from Caltag. Analyses were performed on a FACSCAN flow cytometer (Becton-Dickinson) using FACSCAN research software. HPB.45.0 (CD45-negative) and HPB.45.1 (CD45-positive) are clones obtained after limiting dilution of HPB-ALL. HPB.45.0 was transfected with cDNAs for murine CD45 (ref. 21) or HM1 (ref. 16) in expression vectors, by electroporation and selection for G418 resistance as described¹⁶, generating HPB.45.0.m45 and HPB.45.0.HM1. J-HM1-2.2 is a Jurkat derivative expressing HM1 (ref. 16).

we transfected the CD45-negative HPB.45.0 cell line with a murine complementary DNA clone encoding CD45 (ref. 21). The intracellular portions of murine and human CD45, including the tyrosine phosphatase regions, are 85% homologous²². Although we have been unable to attain levels of murine CD45 expression comparable to levels of human CD45 observed in the CD45-positive HPB.45.1, several transfected clones do express low levels of murine CD45. Of these, several independent clones have been tested for responsiveness to anti-CD3 mAb. In each case, CD45-positive clones such as HPB.45.0 m45 (Fig. 2d; surface staining characteristics shown in Fig. 1g, h) show a significantly greater increase in $[Ca^{2+}]_i$ than does the CD45-negative HPB.45.0 host (Fig. 2a). Thus, even at low levels of expression of murine CD45, substantial restoration of the $[Ca^{2+}]_i$ response was observed.

To begin to localize the site of action of CD45 in regulation of the PtdIns second messenger pathway in human T cells, we examined the function of a heterologous receptor, the human muscarinic receptor type 1 (HM1), in T cells which do not express CD45. This receptor, which is not expressed in human T cells, can activate the PtdIns pathway^{15,16,23}. HPB.45.0 was transfected with HM1 and a positive line, HPB.45.0.HM1, was identified by ³H-quinuclidinyl benzylate (a muscarinic antagonist) binding (data not shown). This cell continued to express the TCR and failed to express CD45 (Fig. 1i, j). The ability of the transfectant to increase $[Ca^{2+}]_i$ in response to stimulation of both HM1 and the TCR was compared with the non-transfected parent and to a Jurkat line transfected with HM1, J-HM1-2.2, which is TCR- and CD45-positive (Fig. 1k, l). Neither the

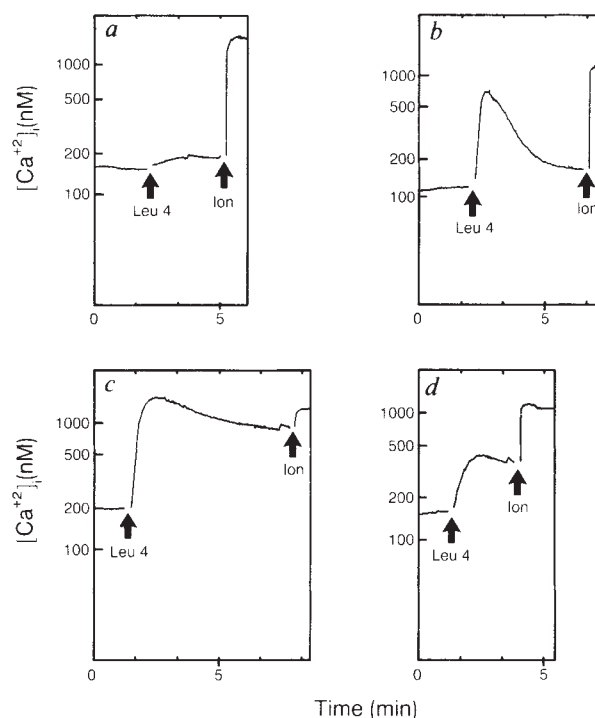


FIG. 2 $[Ca^{2+}]_i$ after stimulation of CD45-negative and CD45-positive derivatives of HPB-ALL. HPB.45.0, CD45-negative (a), HPB.45.1, CD45-positive (b), HPB.45.0 treated with PMA (50 ng ml⁻¹ for 48 h, CD45-positive (c), and HPB.45.0.m45, murine CD45-positive (d).

METHODS. Each cell type was loaded with 3 μ M Indo-1 (Molecular Probes) for 40 min as described²⁷. Real time fluorescence was measured at 37 °C with excitation at 334 nm and emission detection at 400 nm in a spectrofluorimeter (Spex Industries). Anti-CD3 mAb (Leu 4) ascites was added (arrow) at a final concentration of 1:1,000 (a saturating amount), ionomycin (Calbiochem) was added at a final concentration of 1 μ M. Maximal fluorescence was obtained after lysis of the cells with Triton X-100, minimal fluorescence was obtained after chelation with EGTA. $[Ca^{2+}]_i$ was determined as described²⁸.

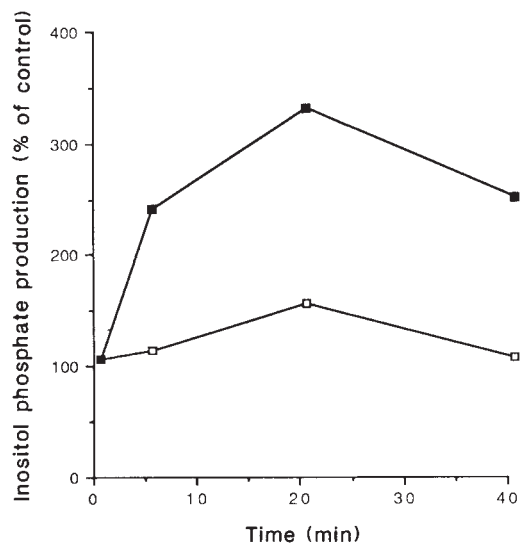


FIG. 3 Time course of soluble inositol phosphate generation in the CD45-negative (□) and CD45-positive (■) cell following TCR stimulation. METHODS. HPB.45.0 (CD45-negative) and HPB.45.1 (CD45-positive) were loaded with [^3H]myo-inositol (Amersham), $40 \mu\text{Ci ml}^{-1}$ for 3 h in phosphate buffered saline then cultured in RPMI 1640 with 10% FCS (16 h) (ref. 16). The cells were then stimulated with anti-CD3 mAb (Leu 4 ascites, 1:1,000 final concentration) in the presence of 10 mM LiCl. At various time points the cells were lysed and soluble inositol phosphates extracted as described¹⁶. Data points represent means of duplicates.

muscarinic agonist, carbachol, nor the anti-CD3 mAb elicited a calcium response in the untransfected HPB.45.0 (data not shown). Carbachol induced a brisk calcium increase in J-HM1-2.2, that was reversed with the muscarinic antagonist, atropine. The cells were fully capable of then responding to stimulation via the TCR, as illustrated by the calcium increase seen after addition of mAb (Fig. 4a). Figure 4b shows that the CD45-negative cell which expresses HM1 also responds to carbachol with a rapid increase in $[\text{Ca}^{2+}]_i$, that was antagonized by atropine. Addition of activating mAb to the TCR, however, elicited no increase in $[\text{Ca}^{2+}]_i$. Thus, expression of HM1 did not restore TCR-mediated signal transduction function. Assays for the generation of soluble inositol phosphates in these cells resulted in comparable results, such that stimuli inducing $[\text{Ca}^{2+}]_i$ increases also elicited increases in inositol phosphates (data not shown).

These data indicate that effective coupling of the TCR to the PtdIns second messenger pathway requires the presence of CD45. The action of CD45 seems to be specific to the TCR activation of the PtdIns second messenger pathway, because in the absence of CD45 the HM1 receptor is fully functional. Although the exact role of CD45 remains to be identified, several studies suggest that this molecule is important in lymphocyte activation¹¹⁻¹⁴. Additionally, tyrosine phosphatase activity of CD45 suggests potential interactions with the ζ -chain of the

CD3 complex which becomes tyrosine-phosphorylated upon T-cell activation²⁴ or with Lck, a T-cell-specific tyrosine kinase of the *Src* family whose activity is modified by the presence or absence of CD45 (refs 9, 10). These results provide a potential link between the tyrosine kinase and PtdIns second messenger pathways associated with the TCR in human T cells. Our data, combined with a report of signalling-deficient T cells that are constitutively phosphorylated on CD3 (ref. 25), suggest that CD45 may have an essential role in the control of ζ -chain phosphorylation, which in turn regulates the ability of the TCR to activate the PtdIns pathway in response to stimuli.

Note added in proof: We have recently isolated CD45-negative mutants of the Jurkat T-cell line which are matched for TCR levels when compared with wild-type cells. These CD45-negative cells also fail to increase PtdIns-derived second messengers when stimulated via the TCR. □

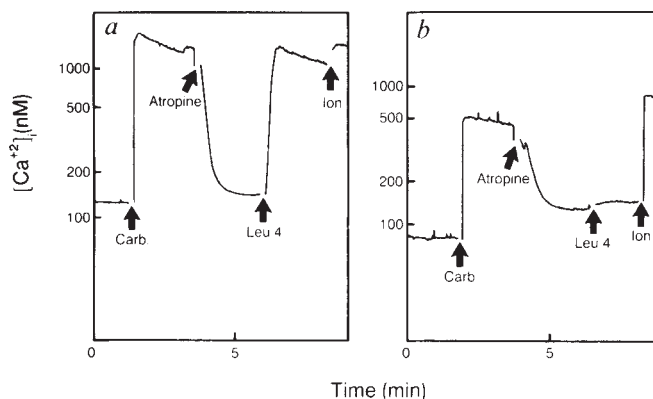


FIG. 4 $[\text{Ca}^{2+}]_i$ levels seen after stimulation of the HM1 or TCR in J-HM1-2.2 (a) and HPB.45.0.HM1 (b). The assay was performed as in Fig. 2. Carbachol (Sigma) was used at a final concentration of $500 \mu\text{M}$ and atropine (Sigma) was used at a final concentration of $10 \mu\text{M}$.

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- Patel, M. D., Samelson, L. E. & Klausner, R. D. *J. Biol. Chem.* **262**, 5831-5838 (1987).
- Samelson, L. E. & Patel, M. D. *Cell* **46**, 1083-1090 (1986).
- Weiss, A. & Imboden, J. B. *Adv. Immun.* **41**, 1-38 (1987).
- Imboden, J. B., Weiss, A. & Stobo, J. D. *J. Immun.* **134**, 663-665 (1985).
- Imboden, J. B. & Stobo, J. D. *J. exp. Med.* **161**, 446-456 (1985).
- Thomas, M. L. *A. Rev. Immun.* **7**, 339-369 (1989).
- Tonks, N. K., Charbonneau, H., Diltz, C. D., Fischer, E. H. & Walsh, K. A. *Biochemistry* **27**, 8695-8701 (1988).
- Kiener, P. A. & Mittler, R. S. *J. Immun.* **143**, 22-28 (1989).
- Mustelin, T., Coggeshall, K. M. & Altman, A. *Proc. natn. Acad. Sci. U.S.A.* **86**, 6302-6306 (1989).
- Ostergaard, H. L. et al. *Proc. natn. Acad. Sci. U.S.A.* **86**, 8959-8963 (1989).
- Pingel, J. T. & Thomas, M. L. *Cell* **58**, 1055-1065 (1989).
- Martorell, J., Vilella, R., Borche, L., Rojo, I. & Vives, J. *Eur. J. Immun.* **17**, 1447-1451 (1987).
- Mittler, R. S., Greenfield, R. S., Schacter, B. Z., Richard, N. F. & Hoffmann, M. K. *J. Immun.* **138**, 3159-3166 (1987).
- Ledbetter, J. A., Tonks, N. K., Fischer, E. H. & Clark, E. A. *Proc. natn. Acad. Sci. U.S.A.* **85**, 8628-8632 (1988).
- Peralta, E. G., Ashkenazi, A., Winslow, J. W., Ramachandran, J. & Capon, D. J. *Nature* **334**, 434-437 (1988).
- Goldsmith, M. G., Desai, D. M., Shultz, T. & Weiss, A. *J. Biol. Chem.* **264**, 17190-17197 (1989).
- Lanier, L. L., Ruitenberg, J. J., Allison, J. P. & Weiss, A. *J. Immun.* **137**, 2286-2292 (1986).
- Oetting, H. C., Terhorst, C., Cantley, L. C. & Rosoff, P. M. *Cell* **40**, 583-590 (1985).
- Berridge, M. J. & Irvine, R. F. *Nature* **312**, 315-321 (1984).
- Akbar, A. N., Terry, L., Timms, A., Beverley, P. C. L. & Janossy, G. *J. Immun.* **140**, 2171-2178 (1988).
- Thomas, M. L., Reynolds, P. J., Chain, A., Ben-Neriah, Y. & Trowbridge, I. S. *Proc. natn. Acad. Sci. U.S.A.* **84**, 5360-5363 (1987).
- Johnson, N. A., Meyer, C. M., Pingel, J. T. & Thomas, M. L. *J. Biol. Chem.* **264**, 6220-6229 (1989).
- Nathanson, N. M. A. *Rev. Neurosci.* **10**, 195-236 (1987).
- Baniyash, M., Gardia-Morales, P., Bonifacio, J. S., Samelson, L. E. & Klausner, R. D. *J. Biol. Chem.* **263**, 9874-9878 (1988).
- Samelson, L. E., Davidson, W. F., Morse, H. C. & Klausner, R. D. *Nature* **324**, 674-676 (1986).
- Weiss, A. & Stobo, J. D. *J. exp. Med.* **160**, 1284-1299 (1984).
- Goldsmith, M. G. & Weiss, A. *Proc. natn. Acad. Sci. U.S.A.* **84**, 6879-6883 (1987).
- Tsien, R. Y., Pozzan, T. & Rink, T. J. *J. Cell Biol.* **94**, 925-934 (1982).

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Hyperpolarization and relaxation of arterial smooth muscle caused by nitric oxide derived from the endothelium

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STIMULATION of the endothelial lining of arteries with acetylcholine results in the release of a diffusible substance that relaxes and hyperpolarizes the underlying smooth muscle¹⁻⁹. Nitric oxide (NO) has been a candidate for this substance, termed endothelium-derived relaxing factor^{10,11}. But there are several observations that argue against the involvement of NO in acetylcholine-induced hyperpolarization. First, exogenous NO has no effect on the membrane potential of canine mesenteric arteries⁷. Second, although haemoglobin (believed to bind and inactivate NO (refs 11-15)) and methylene blue (which prevents the stimulation of guanylate cyclase¹¹⁻¹⁶) inhibit relaxation^{7,12-14}, neither has an effect on hyperpolarization^{5,7,8}. Finally, nitroprusside, thought to generate NO in vascular smooth muscle^{14,16}, relaxes rat aorta without increasing rubidium efflux¹⁷. Nevertheless, nitrovasodilators, nitroprusside and nitroglycerine cause hyperpolarization in some arteries¹⁸⁻²⁰. NO might therefore be responsible for at least part of the hyperpolarization induced by acetylcholine. We now report that hyperpolarization and relaxation evoked by acetylcholine are reduced by *N*^G-monomethyl-L-arginine, an inhibitor of NO biosynthesis from L-arginine²¹⁻²⁵. Thus NO derived from the endothelium can cause hyperpolarization of vascular smooth muscle, which might also contribute to relaxation by closing voltage-dependent calcium channels. Our findings raise the possibility that hyperpolarization might be a component of NO signal transduction in neurons or inflammatory cells.

Ring segments of guinea-pig uterine artery were prepared for simultaneous recording of membrane potential and tension development. Phenylephrine (10^{-8} – 10^{-5} M) was used to depolarize the membrane and to raise tension (Fig. 1a). Infusion of NO caused hyperpolarization and relaxation (Fig. 1b). Concentration–response curves to NO-induced (3.6×10^{-8} M– 3.6×10^{-5} M) hyperpolarization and relaxation were constructed for segments depolarized by 6 ± 0.5 mV ($n = 25$; Fig. 2). The effector concentration for half-maximum response (EC_{50}) for NO was 1.0×10^{-6} M for the relaxation, and 2.4×10^{-6} M for the hyperpolarization. If, in some arteries, there was a large difference in the doses at which the two responses occurred, this could explain why other studies have reported relaxation in response to NO without detectable hyperpolarization. In uterine arteries, relaxation occurred at the lowest doses of NO without hyperpolarization. At higher levels of depolarization, the maximum relaxation was always accompanied by hyperpolarization, suggesting that the hyperpolarization makes a greater contribution to relaxation when calcium influx is high.

Because NO can evoke hyperpolarization in the uterine artery, we examined whether NO of endothelial origin might account for some of the hyperpolarization in response to acetylcholine. Hyperpolarization and relaxation of uterine arteries in response to acetylcholine were both reduced to a similar extent by *N*^G-monomethyl-L-arginine, NMMA (2×10^{-5} M, Fig. 3), which reduces endothelium-dependent relaxation in blood vessels^{22,23,25}. NMMA significantly reduced hyperpolarization

and relaxation of uterine arteries by $41 \pm 7\%$ and $50 \pm 6\%$, respectively ($n = 10$; Fig. 3b). L-Arginine (10^{-4} M) completely prevented the inhibition caused by NMMA (2×10^{-5} M; Fig. 3c). NMMA also reduced acetylcholine-induced hyperpolarization in mesenteric and coronary arteries of guinea-pigs. Thus, endothelium-derived relaxing factor (EDRF) is capable of

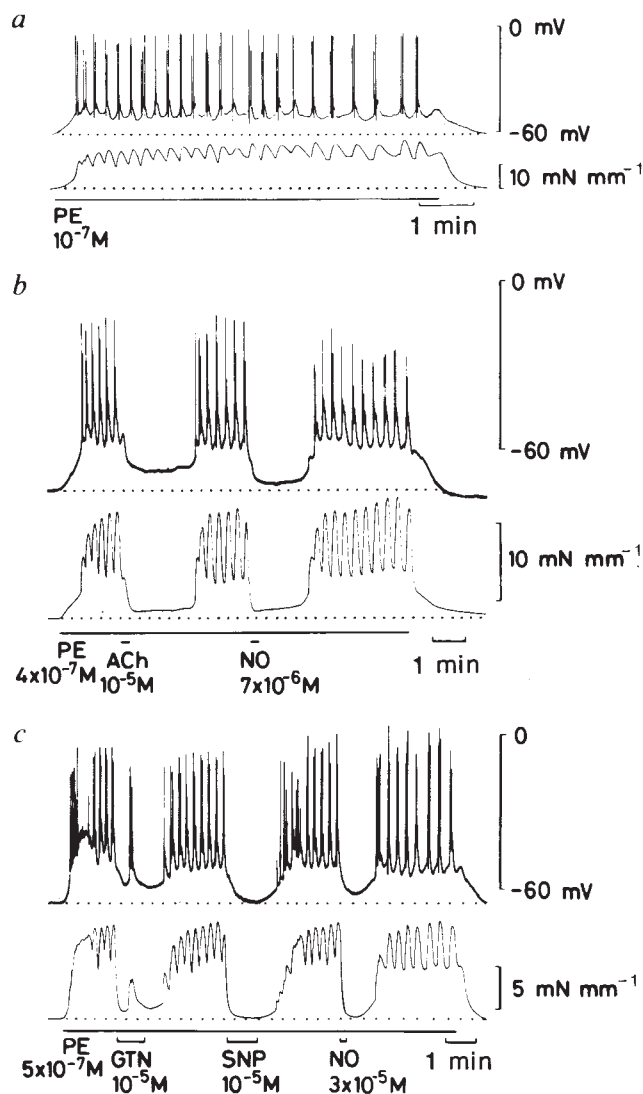


FIG. 1 Acetylcholine, NO and the nitrovasodilators cause hyperpolarization and relaxation of uterine artery. *a*, When the depolarization (upper trace) in response to phenylephrine (PE) was in the range -52 ± 1 mV and -41 ± 1 mV ($n = 22$), oscillations occurred in membrane potential, often topped with action potentials. Each oscillation was followed by a phasic increase in tension (lower trace). *b*, Application of acetylcholine to a vessel depolarized (upper trace) and constricted (lower trace) caused hyperpolarization and relaxation and these responses were mimicked by NO. *c*, Glyceryl trinitrate (GTN) and sodium nitroprusside (SNP) evoked hyperpolarization (upper trace) and relaxation (lower trace) that were similar to those caused by NO. **METHODS.** Ring segments (1–2 mm in length) of uterine arteries from guinea-pigs were mounted on a Mulvany–Halpern-style myograph²⁹ with two stainless steel wires 40 μ m in diameter, and continuously perfused with physiological solution containing: NaCl, 120 mM; KCl, 5 mM; KH_2PO_4 , 1 mM; $NaHCO_3$, 25 mM; $MgSO_4$, 1.2 mM; $CaCl_2$, 2.5 mM; and glucose, 11 mM; gassed with 95% O_2 , 5% CO_2 and maintained at 35 °C. Each segment was stretched in increments until the tension was equivalent to 60–65 mmHg. Membrane potential was measured simultaneously. Solutions of NO were made from NO gas dissolved in deoxygenated water and introduced into the perfusion line with a delay of 1–2 s between the point of injection and the preparation.

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TABLE 1 Effect of membrane potential on hyperpolarization and relaxation in uterine arteries of guinea-pigs

Resting membrane potential (mV)	Membrane potential (mV) in phenylephrine	% Hyperpolarization		% Relaxation	
		ACh (10^{-5} M)	NO (2.5×10^{-5} M)	ACh (10^{-5} M)	NO (2.5×10^{-5} M)
-69 ± 1 (41)	-64 ± 1 (23)	84 ± 10 (13)	114 ± 11 (10)	118 ± 12 (13)	126 ± 5 (10)
	-29 ± 1 (16)	13 ± 5 (9)	27 ± 4 (7)	14 ± 4 (9)	40 ± 8 (7)

Arteries were exposed to phenylephrine (5×10^{-8} to 5×10^{-7} M) and (5×10^{-7} to 5×10^{-5} M) that depolarized the smooth muscle cells to -64 mV and -29 mV, respectively. Hyperpolarization and relaxation are expressed as percentages of the depolarization and constriction, respectively, induced by phenylephrine. Values of n are given in parentheses. ACh, acetylcholine.

contributing to this hyperpolarization in these vessels. Because both hyperpolarization and relaxation were not completely abolished, it is possible that another mechanism contributes to the hyperpolarization and relaxation in response to acetylcholine.

Exposure of uterine arteries to methylene blue (10^{-5} M) for 15–20 min before addition of phenylephrine caused depolarization and tension development. In the presence of methylene blue and phenylephrine, the concentration–response curves to NO for both hyperpolarization and relaxation were shifted to the right. After prolonged exposure to methylene blue, both hyperpolarization and relaxation were abolished (Fig. 2a, b). Both hyperpolarization and relaxation in response to acetylcholine were reduced or abolished after incubation in methylene blue ($n = 5$). Exposure to 10 μ M haemoglobin for 10 min before and during application of phenylephrine resulted in abolition of NO-induced hyperpolarization and relaxation (Fig. 2a, b). Haemoglobin also reduced relaxation in response to acetylcholine in each of seven preparations. In three of these the hyperpolarization was also reduced, but it remained unchanged in the remaining four.

The ability of NO to hyperpolarize uterine arteries might have reflected properties unique to these arteries, but we found that NO hyperpolarized and relaxed all other vessels studied: phenylephrine-depolarized mesenteric ($n = 12$), mammary ($n = 3$) and saphenous ($n = 1$) arteries and aorta ($n = 1$) of guinea-pigs; tail arteries from rats ($n = 3$); saphenous ($n = 1$) and femoral ($n = 1$) arteries from rabbit; mesenteric arteries from dogs ($n = 2$) and guinea-pig coronary arteries at rest ($n = 15$). The ability of some arteries to respond to NO declined with repeated application. That is, there was tachyphylaxis of both the hyperpolarization and relaxation, and the hyperpolarization usually disappeared before the relaxation.

Nitroprusside and nitroglycerine are thought to cause relaxation by liberating NO in smooth muscle cells^{14,16}. These nitrovasodilators evoke hyperpolarization in some vascular smooth muscles^{18–20} but are without effect on others^{17,26–28}. In six uterine arteries depolarized by 7 ± 1 mV and constricted by 1.7 ± 0.3 mN mm⁻¹ with phenylephrine (8×10^{-8} – 2×10^{-6} M), sodium nitroprusside (10^{-5} M) and nitroglycerine (10^{-5} M) caused hyperpolarization of $138 \pm 16\%$ and $110 \pm 8\%$, respectively, accompanied by relaxation of $128 \pm 9\%$ and $117 \pm 8\%$, respectively (Fig. 1c). In contrast to the actions of acetylcholine, the actions of NO and the nitrovasodilators were independent of the integrity of the endothelium. The nitrovasodilators could hyperpolarize rat tail arteries, but only in those preparations in which the resting membrane potential was more depolarized than -70 mV (ref. 19). NO and acetylcholine could not hyperpolarize uterine arteries that were not depolarized by phenylephrine. These arteries have a high negative resting membrane potential (-69 ± 1 mV, $n = 41$), compared with mesenteric and coronary arteries (-61 ± 2 mV ($n = 12$) and -58 ± 2 mV ($n = 15$), respectively), and in none of these arteries could acetylcholine or NO hyperpolarize the membrane beyond about -70 mV.

The hyperpolarization in response to acetylcholine is due to an increase in potassium conductance^{2,3,8}. When the potassium concentration in the perfusing solution was raised (10–100 mM), the hyperpolarization induced by both acetylcholine and NO in uterine arteries was reduced or abolished. Others have shown that the ability of acetylcholine to induce hyperpolarization and

relaxation is dependent on the extent of depolarization elicited by the raised external potassium. At large depolarizations, there is a reduction in amplitude of both the hyperpolarization^{2,8} and the relaxation¹⁷. It can be seen from Table 1 that the hyperpolarization and relaxation induced by acetylcholine (10^{-5} M) and NO (2.5×10^{-5} M) are also dependent on the extent of depolarization induced by phenylephrine in uterine arteries. Thus, there is a range of membrane potential in which NO can

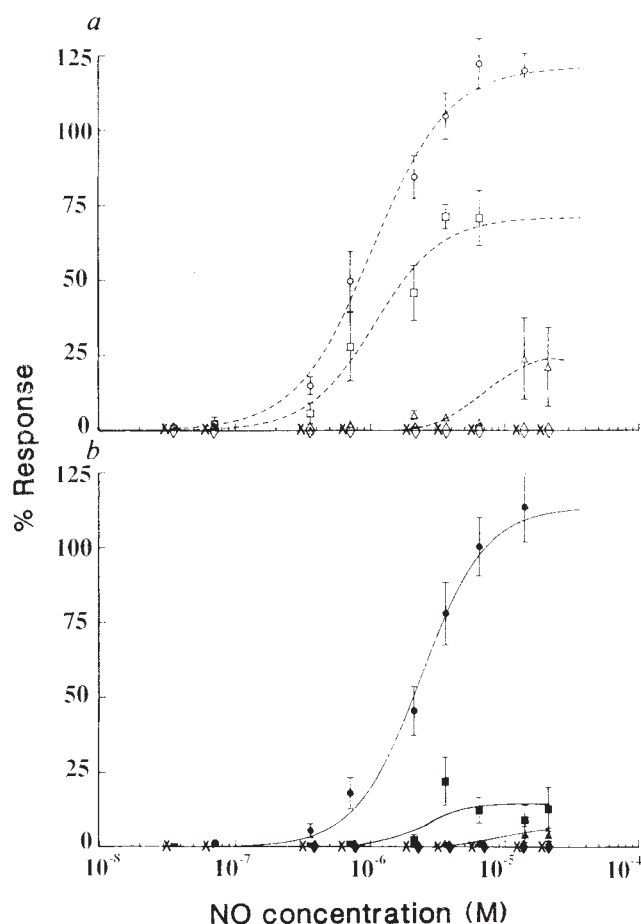


FIG. 2 Dose-dependent hyperpolarization of the membrane and relaxation evoked by NO in uterine arteries of guinea-pigs. a, Relaxation (open symbols). b, Hyperpolarization (filled symbols). The arteries were exposed to concentrations of phenylephrine (10^{-8} – 5×10^{-6} M) that depolarized the smooth muscle by 6 ± 0.5 mV and raised tension by 4 ± 0.4 mN mm⁻¹. Concentrations of NO (4×10^{-8} – 4×10^{-5} M) were applied and the hyperpolarization and relaxation that resulted expressed as a percentage of the phenylephrine-induced depolarization and increase in tension, respectively. NO evoked concentration-dependent relaxation (a) and hyperpolarization (b) in uterine arteries (circles; $n = 24$). Methylene blue (10^{-5} M) caused time-dependent inhibition of both relaxation ($n = 10$) and hyperpolarization ($n = 9$; squares, 21 min; triangles, 53 min; diamonds, 81 min). Both responses were abolished after 81 min. Exposure to haemoglobin (10^{-5} M) abolished the responses (crosses; $n = 7$). Vertical bars represent standard errors of the mean. The number of animals studied (n) (not the number of cells impaled) is used throughout.

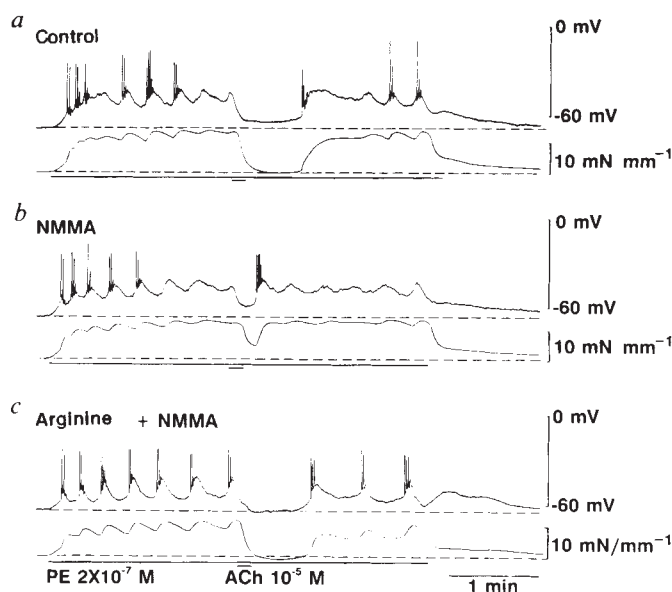


FIG. 3 Inhibition of acetylcholine-induced hyperpolarization and relaxation by L-NMMA. *a*, Hyperpolarization (upper trace) and relaxation (lower trace) evoked by 10^{-5} M acetylcholine in a uterine artery depolarized and constricted with phenylephrine (PE). *b*, Preincubation of the tissues with NMMA acetate (2×10^{-5} M) reduced the amplitude and duration of the hyperpolarization and relaxation in response to 10^{-5} M acetylcholine. *c*, The effect of this concentration of NMMA was antagonized by 10^{-4} M L-arginine, added during and for 10 min before application of NMMA.

cause hyperpolarization. The more negative potentials are close to the equilibrium potential for potassium, whereas at substantial levels of depolarization, the magnitude of total conductance swamps NO-induced potassium conductances. The level of membrane potential in determining the ability of acetylcholine and NO to elicit hyperpolarization may explain why others have failed to find an effect of NO on membrane potential. Moreover, in our study membrane potential and developed tension have been measured simultaneously in the same tissue.

In conclusion, NO, whether derived from the endothelium as EDRF or from nitrovasodilator drugs, can mediate hyperpolarization of arterial smooth muscle. Although NO can induce relaxation by stimulation of calcium extrusion alone, the contribution of the hyperpolarization to relaxation is probably more important when calcium influx is high. □

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- Furchgott, R. F. & Zawadzki, J. V. *Nature* **288**, 373–376 (1980).
- Kuriyama, H. & Suzuki, H. *Br. J. Pharmacol.* **64**, 493–501 (1978).
- Bolton, T. B., Lang, R. J. & Takewaki, T. *J. Physiol., Lond.* **351**, 549–572 (1984).
- Komori, K. & Suzuki, H. *Br. J. Pharmacol.* **92**, 657–664 (1987).
- Chen, G., Suzuki, H. & Weston, A. H. *Br. J. Pharmacol.* **95**, 1165–1174 (1988).
- Feletou, M. & Vanhoutte, P. M. *Br. J. Pharmacol.* **93**, 515–524 (1988).
- Komori, K., Lorenz, R. R. & Vanhoutte, P. M. *Am. J. Physiol.* **255**, H207–212 (1988).
- Chen, G. & Suzuki, H. *J. Physiol., Lond.* **410**, 91–106 (1989).
- Parkington, H. C. *et al.* *Proc. Aust. Physiol. Pharmac. Soc.* **20**, 17P (1989).
- Palmer, R. M. J., Ferrige, A. G. & Moncada, S. *Nature* **327**, 524–526 (1987).
- Ignarro, L. J., Buga, G. M., Wood, K. S., Byrns, R. E. & Chaudhuri, G. *Proc. natn. Acad. Sci. U.S.A.* **84**, 9265–9269 (1987).
- Dusting, G. J., Read, M. A. & Stewart, A. G. *Clin. exp. Pharmac. Physiol.* **15**, 83–92 (1988).
- Martin, W., Villani, G. M., Jothianandan, D. & Furchgott, R. F. *J. Pharmac. exp. Ther.* **232**, 708–716 (1985).
- Ignarro, L. J. & Kadowitz, P. J. *Rev. Pharmac. Tox.* **25**, 171–191 (1985).
- Feilisch, M. & Noack, E. A. *Eur. J. Pharmacol.* **139**, 19–30 (1987).
- Waldman, S. A. & Murad, F. *Pharmac. Rev.* **39**, 163–196 (1987).
- Taylor, S. G., Southerton, J. S., Weston, A. H. & Baker, J. R. J. *Br. J. Pharmacol.* **94**, 853–863 (1988).
- Itoh, Y., Suzuki, H. & Kuriyama, H. *J. Pharmac. exp. Ther.* **207**, 1022–1031 (1978).
- Cheung, D. W. & Mackay, M. J. *Br. J. Pharmacol.* **86**, 117–124 (1985).
- Itoh, Y., Kitamura, K. & Kuriyama, H. *Br. J. Pharmacol.* **70**, 197–204 (1980).
- Palmer, R. M. J., Ashton, D. S. & Moncada, S. *Nature* **333**, 664–666 (1988).
- Palmer, R. M. J., Rees, D. D., Ashton, D. S. & Moncada, S. *Biochem. biophys. Res. Commun.* **153**, 1251–1256 (1988).
- Sakuma, I., Stuehr, D. J., Gross, S. S., Nathan, C. & Levi, R. *Proc. natn. Acad. Sci. U.S.A.* **85**, 8664–8667 (1988).
- Palmer, R. M. J. & Moncada, S. *Biochem. biophys. Res. Commun.* **158**, 345–352 (1989).

- Rees, D. D., Palmer, R. M., Hodson, H. F. & Moncada, S. *Br. J. Pharmacol.* **96**, 418–424 (1989).
- Itoh, Y., Kitamura, H. & Kuriyama, H. *J. Physiol., Lond.* **309**, 171–183 (1980).
- Beny, J. L. & Burnet, P. C. *Blood Vessels* **25**, 308–311 (1988).
- Huang, A. H., Busse, R. & Bassenge, E. *Naunyn-Schmiedeberg, Archs Pharmac.* **338**, 438–442 (1988).
- Mulvany, M. J. & Halpern, W. *Circulation Res.* **41**, 19–26 (1977).

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Subsecond deactivation of transducin by endogenous GTP hydrolysis

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THE response of a retinal rod cell to a weak flash of light is mediated by a receptor/GTP-binding protein (rhodopsin/transducin) signal transduction system^{1,2} and terminates within a second³. The $T\alpha$ subunit of transducin (composed of subunits $T\alpha$, $T\beta$ and $T\gamma$) is triggered by photoexcited rhodopsin (R^*) to release GDP and bind GTP. The binding of GTP causes release of the $T\alpha$ unit from $T\beta\gamma$ and allows it to modulate the activity of an enzyme that generates a second messenger. Termination of the response requires the hydrolysis of the GTP by intrinsic GTPase^{4,5}. As with other G proteins, the GTPase activity of transducin seems to be slow. Reported *in vitro* turnover rates of a few molecules of GTP hydrolysed per molecule of transducin per minute^{6–9} imply a $T\alpha$ -GTP deactivation time of many seconds. But this time might be only a small fraction of that of the GTPase cycle. We have now used time-resolved microcalorimetry^{10–12} in bovine rod outer segments (ROS) to monitor the heat release due to the hydrolysis of GTP by a transducin population that had been quickly activated by flash illumination of rhodopsin. The enthalpy of GTP hydrolysis is released within 1 s at 23 °C. This deactivation time seems to be independent of any diffusible factor in the preparation and concurs with the termination kinetics of the rod's response. Thereafter, transducin seems unable to reload GTP for many seconds. This refractory 'resetting' time may account for the low steady-state GTPase rates *in vitro*.

In vitro steady-state measurements of the GTPase rate of transducin under continuous illumination (a condition for receptor saturation) assay a complete cycle that includes¹³ encounter of $T\alpha$ -GDP- $T\beta\gamma$ with photoexcited rhodopsin, R^* -catalysed GDP/GTP exchange on $T\alpha$, dissociation of active $T\alpha$ -GTP from R^* and $T\beta\gamma$, deactivation by endogenous GTP hydrolysis, and reassociation of $T\alpha$ -GDP with $T\beta\gamma$ before a new cycle can begin. The first three steps determine the millisecond activation time of transducin¹⁴, whereas the hydrolytic step determines the deactivation time. Any other steps such as a long-lived post-hydrolytic GDP-Pi conformation (as with tubulin or actin) or a slow re-association of $T\alpha$ -GDP with $T\beta\gamma$ will lengthen the GTPase cycle.

Whereas steady-state techniques necessarily give a sum total of these times, they may be separated by a time-resolved technique applied to a transiently activated sample.

Under weak flash illumination, fast quenching of R^* is achieved *in vivo* by the combined action of rhodopsin kinase and arrestin¹⁵, the latter taking <0.2 s to bind to phosphorylated R^* (ref. 16). *In vitro*, this quenching takes seconds even with kinase and arrestin enrichment of concentrated ROS suspensions^{15,17}. If needed, however, hydroxylamine can be used

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to accelerate the deactivation of R^* by displacing its retinal¹⁸.

Most of the enthalpy of GTP hydrolysis is released in three steps: R^* -catalysed GTP loading and associated transconformation of $T\alpha$; hydrolysis of $T\alpha$ -GTP to $T\alpha$ -GDP per se; and reassociation of $T\alpha$ -GDP with $T\beta\gamma$. So the kinetics of this enthalpy release constitutes an upper-bound of the $T\alpha$ -GTP lifetime. We assessed the enthalpy release using a custom-designed time-resolved microcalorimeter (Fig. 1), on flash-illuminated ROS suspensions. With the addition of both GTP and cyclic GMP (Fig. 2a) there arises a large signal resulting mainly from the amplified hydrolysis of cGMP by the activated cGMP phosphodiesterase (cGMP-PDE). Hydrolysis of known amounts of cGMP provides an internal calibration of the cell's thermal sensitivity (Fig. 2b). With the addition of GTP only, a smaller signal due to light-induced GTP hydrolysis (Fig. 3a) is observed. It follows a fast spike due to heating by the absorbed light flash that is also seen alone in the absence of GTP or after its exhaustion (Fig. 3c). The signal with only GTP present is not due to contaminant cGMP as the preparations used have in the dark a nonspecific PDE¹⁹ that is more active than the guanylate cyclase (Fig. 2b).

The GTP hydrolysis signal almost saturates at $R^*/R = 2.6 \times 10^{-4}$ (Fig. 3b), where the integrated amplitude of the signal

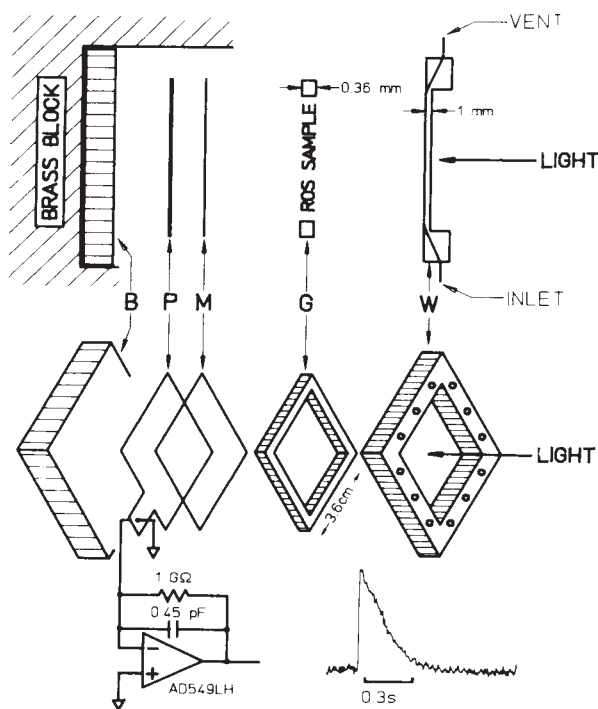


FIG. 1 The active element of the microcalorimeter is a 40- μ m-thick poled, polyvinylidene fluoride (PVDF)²⁵ film gold-plated on both faces (P) (Solvay & Cie, Bruxelles). The back electrode is epoxy-glued to a thermally insulating slab (10-mm thick) of balsa wood (B) and the front electrode is protected from the liquid sample by a 6- μ m mylar layer (M). A 0.36-mm-thick Parafilm gasket (G) and a Teflon window (W) define a 3.6 $\times$ 3.6-cm sample chamber. The film's grounded front electrode acts as a shield to the back electrode which is connected to the operational amplifier. The cell thus arranged is adiabatic. It is mounted together with the amplifier between two 15-kg blocks of brass (S) stabilized at 23 °C in a styrofoam box; the block in front has a double-paned window through which the sample can be illuminated. This instrument is an improved version of one described before³¹. Dilution from heating causes the PVDF polarizability to decrease, generating a displacement current proportional to the derivative of the film temperature²⁵. The integral of this signal is proportional to the quantity of heat received by the film. This device thus monitors in real time the rate of reactions through their enthalpy release. The trace shown is the instrument's response obtained by flashing a dye solution. Direct heating of the PVDF by the light flash was accounted for by subtraction using the same procedure as in ref. 12.

roughly corresponds to one turnover of the transducin pool (using a ΔH value of 5–6 kcal mol⁻¹ for GTP hydrolysis, based on the ΔG value of 7.3 kcal mol⁻¹ for ATP hydrolysis). At low levels of NH_2OH this hydrolysis signal displays a fast pulse component and a slow long tail (Fig. 3a, g). The rise time of the pulse component is about 0.3 s, its fall time about 0.6 s. With only 5 mM NH_2OH , R^* lasts 6–10 s¹⁸ during which it continuously catalyses the turnover of transducin. The pulse component in Fig. 3a thus corresponds to the first round of GTP hydrolysis by all the transducin that is ready before the light flash: immediately after illumination and once only, transducin is quickly activated and almost synchronously deactivated by GTP hydrolysis. Subsequently, and as long as some R^* persists, transducin turns over. The rate of the heat release is then governed by the duration of the whole cycle which must include one or many slow steps as the long tail in Fig. 3a is much smaller than the pulse. Thus, if there is little NH_2OH , and some R^* persists, transducin will recycle but the GTPase turnover rate will be limited by the slow 'resetting' steps. One might postulate a long-lived intermediate refractory $T\alpha$ ($T\alpha_{ref}$) that still contains the γ -phosphate and/or is still soluble like $T\alpha$ -GTP and thus cannot reassociate with $T\beta\gamma$. With 25 mM NH_2OH (Fig. 3d), one expects R^* to decay faster, resulting in little or no transducin turnover and a concomitant reduction or disappearance of the tail component, which is indeed the case. As the R^* lifetime should not kinetically influence transducin activation and

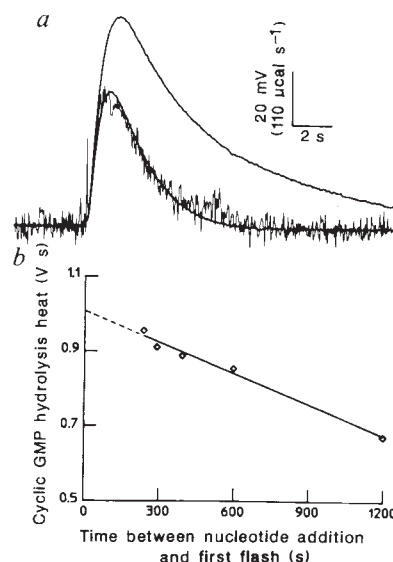


FIG. 2 Cyclic GMP hydrolysis signals and microcalorimeter calibration. a, Flash-induced cGMP hydrolysis in dilute ROS samples: 3.8 μ M rhodopsin, 0.2 mM GTP, 3 mM cGMP, 0.5 mM NH_2OH (upper trace), 50 mM NH_2OH (lower thick trace) or 200 mM NH_2OH (lower thin trace, signal $\times 14$ to normalize to that with 50 mM NH_2OH), flash photoexcitation $R^*/R = 5.2 \times 10^{-5}$. b, Calibration by cGMP hydrolysis in dilute samples (composition and photoexcitation as in a, lower thick trace). As there is a nonspecific PDE activity in the dark¹⁹, samples were illuminated at different times after nucleotide addition by series of 20 flashes to exhaust the cGMP (GTP hydrolysis heat is $< 10^{-3}$ times that of cGMP hydrolysis); the total hydrolysis heat from each sample is the sum of the integrated areas of these 20 signals. Using $\Delta H = 12.3$ kcal mol⁻¹ (ref. 12) for cGMP hydrolysis, zero-time extrapolation of the linear fit through these sums gives a calibration value (1 mV.s = 5.5 μ cal) about equal to that obtained from flash-heating a dye solution.

METHODS. Bovine ROS prepared as in ref. 26 and stored at -70 °C were resuspended into buffer in total darkness and incubated 20 min at 23 °C with 1 mM NH_2OH to scrub R^* formed before or during preparation. This concentrated (150–250 μ M rhodopsin) stock suspension was stored on ice until use. Adequate amounts of buffer, GTP, cGMP, NH_2OH (adjusted with KOH to pH 7.5), were added and the sample (0.46 ml) injected into the cell. Measurements were begun after 4–5 min to allow thermal equilibration with the cell. Buffer composition: 100 mM KCl, 20 mM HEPES, pH 7.5, 2 mM $MgCl_2$, 0.4 mM DTT.

deactivation, the width of the pulse in Fig. 3d is mostly identical to that of Fig. 3a. At still higher NH_2OH levels (50 mM, data not shown), R^* is too short-lived to activate the whole transducin pool: the GTP hydrolysis pulse slightly decreases in amplitude while maintaining the same kinetics. This effect is also seen with the cGMP hydrolysis signal: with an increase in NH_2OH concentration from 0.5 to 50 mM, a long tail disappears, whereas with a change of 50 to 200 mM NH_2OH , signal amplitude decreases but there is no change in kinetics (Fig. 2a).

The kinetics of the GTP hydrolysis pulse is unchanged by a two fold dilution of the sample (Fig. 3e) and therefore seems independent of a soluble accelerating cofactor.

Double-flash experiments reveal a refractory time during which the GTP signal cannot be fully re-elicited. Recovery takes 4–8 s at 50 mM NH_2OH (Fig. 4a–c), more than 12 s at 10 mM NH_2OH (Fig. 4d–e) and 2 min at 2 mM NH_2OH (not shown). A high NH_2OH concentration quickly deactivates R^* , and transducin activation ends soon after the flash. The 4–8-s refractory time is then the resetting time needed to replenish the $\text{T}\alpha\text{-GDP-T}\beta\gamma$ pool before another signal is possible. With little NH_2OH , R^* and transducin activation persist after the flash: turnover occurs, governed by the slow resetting process. Hence, $\text{T}\alpha_{\text{ref}}$ and/or $\text{T}\alpha\text{-GDP}$ accumulate, but the $\text{T}\alpha\text{-GDP-T}\beta\gamma$ pool is hardly replenished: a second flash elicits no signal (Fig. 4d); the longer refractory time at low NH_2OH concentration merely

approximates the R^* lifetime during which $\text{T}\alpha\text{-GDP-T}\beta\gamma$ is not replenished.

The R^* -quenching effect of NH_2OH is not strictly required in demonstrating the fast kinetics of endogenous GTP hydrolysis, as the activation–deactivation times are much faster than the resetting time. This significant kinetic difference allows the separation of the two processes even when there is transducin turnover at low NH_2OH concentrations (Fig. 3a). High NH_2OH concentrations, when used in less crucial experiments, seem relatively harmless. As checked by the pH metric technique²⁰ on continuously illuminated samples, the saturated cGMP-PDE activity is not modified by addition of 50 mM NH_2OH (data not shown). Furthermore, hydroxylamine does not seem to accelerate transducin deactivation, as the GTP hydrolysis pulse does not speed up when the concentration of NH_2OH increases from 5 mM to 25 mM (Fig. 3a, d).

The *in vitro* kinetics of the transducin GTPase cycle thus include three or four steps: (1) activation, $\text{T}\alpha\text{-GDP-T}\beta\gamma \rightarrow \text{T}\alpha\text{-GTP} + \text{T}\beta\gamma$, taking about 0.1 s; (2) deactivation, $\text{T}\alpha\text{-GTP} \rightarrow \text{T}\alpha_{\text{ref}}$, completed within 1 s; (3) regeneration, $\text{T}\alpha_{\text{ref}} \rightarrow \text{T}\alpha\text{-GDP}$; and/or (4) reassociation: $\text{T}\alpha\text{-GDP} + \text{T}\beta\gamma \rightarrow \text{T}\alpha\text{-GDP-T}\beta\gamma$, which may last many seconds.

Being in the subsecond range, endogenous GTP hydrolysis qualifies kinetically as a shut-off mechanism for visual transduction; its speed does not depend on soluble cofactors (contrary to refs 21 and 22), but transducin interaction with native disk membranes and, as suggested for other G proteins, a physiological ionic composition²³, might be crucial. The resetting time during which transducin cannot reload GTP accounts for the reported low steady-state GTPase rates. This could be an *in vitro* effect due partially to the solubilization of $\text{T}\alpha\text{-GTP}$ causing a slow recombination of the resulting $\text{T}\alpha\text{-GDP}$ to membrane-bound $\text{T}\beta\gamma$. Although $\text{G}\alpha\text{-GTP}$ subunits of other G proteins do not seem as soluble as $\text{T}\alpha\text{-GTP}$, their reported GTPase rates *in vitro* might also be poor indicators of their lifetimes *in vivo*.

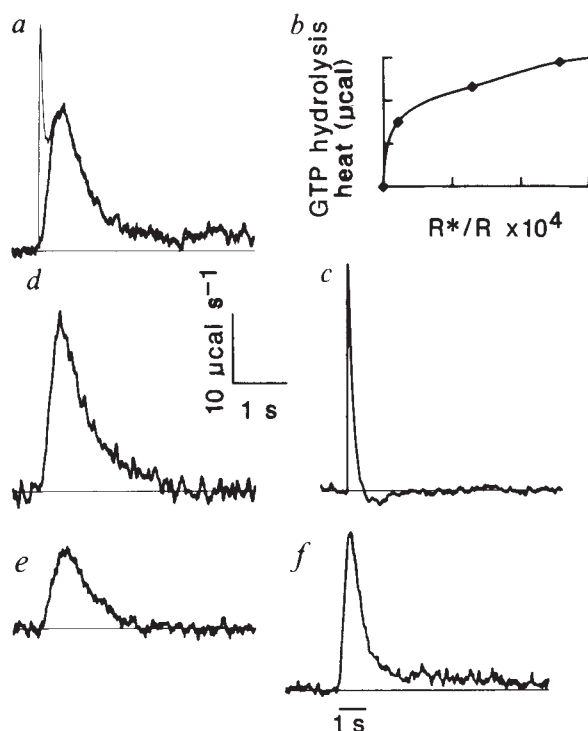


FIG. 3 Kinetics of GTP hydrolysis. *a*, Light-induced GTP hydrolysis signal at low NH_2OH : 63 μM rhodopsin, 2 mM GTP, 5 mM NH_2OH , $R^*/R=1.3 \times 10^{-4}$ (thin trace). The spike preceding the broader signal is due to direct heating of the PVDF film by the light flash. *b*, Integrated areas (in μcal) of GTP hydrolysis signals as a function of photolysis levels: 75 μM rhodopsin, 2 mM GTP, 50 mM NH_2OH , $R^*/R=2.2 \times 10^{-5}$, 1.3×10^{-4} and 2.6×10^{-4} . *c*, Average of eight flash spikes collected after GTP exhaustion; its subtraction from the raw signal reveals the heat generated by the sample itself (a, thick trace). The slight negative component is due to cooling of the PVDF film by the exhausted sample. *d*, *e*, GTP hydrolysis signals at higher NH_2OH from: 75 μM (*d*), 33 μM (*e*) rhodopsin, 2 mM GTP, 25 mM NH_2OH , $R^*/R=1.3 \times 10^{-4}$. *f*, Average of two experiments at low NH_2OH levels (same composition and illumination as in *a*) shown on a $\times 2$ compressed time scale. These GTP hydrolysis signals are repeatable up to 50 times. The signals in *a*, *d*, *e* and *f* come from samples of the same preparation. (The significant light-independent GTPase activity of these ROS samples adds only a constant to the baseline.)

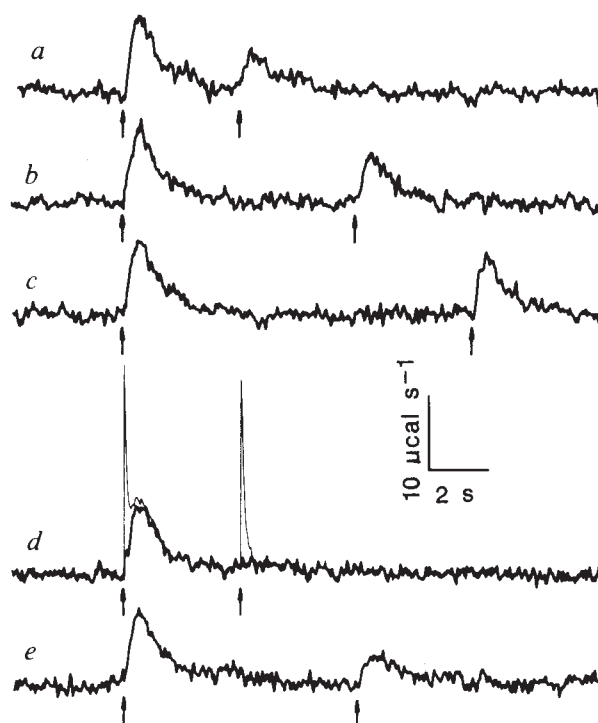


FIG. 4 Double-flash experiments. Varyingly spaced pairs of flashes (arrows, $R^*/R=1.3 \times 10^{-4}$) from two aliquots containing 69 μM rhodopsin, 2 mM GTP, with 50 mM NH_2OH (*a*–*c*), or 10 mM NH_2OH (*d*, *e*). Each trace is an average of four (*a*–*c*) or two (*d*, *e*) recordings separated by 1–2 min. The flash spikes have been subtracted (as in Fig. 3a) except in *d* (thin trace) where both flash spikes can be seen in the unsubtracted data.

which control the duration of effector activation, and hence the integrated intensity of cellular responses. The recent demonstration that a mutated $G\alpha$ with reduced GTPase activity is involved in cellular transformation²⁴ stresses the importance of an accurate knowledge of the true lifetime of $G\alpha$ -GTP. □

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1. Stryer, L. *A. Rev. Neurosci.* **9**, 87–119 (1986).
2. Liebman, P. A. & Pugh, E. N. *Vis. Res.* **19**, 375–380 (1979).
3. Baylor, D. A., Nunn, B. J. & Schnap, J. L. *J. Physiol., Lond.* **357**, 575–607 (1984).
4. Sather, W. A. & Detwiler, P. B. *Proc. natn. Acad. Sci. U.S.A.* **84**, 9290–9294 (1987).
5. Lamb, T. D. & Matthews, J. *J. Physiol., Lond.* **407**, 463–487 (1988).
6. Fung, B. K.-K. *J. biol. Chem.* **258**, 10495–10502 (1983).
7. Yamanaka, G., Eckstein, F. & Stryer, L. *Biochemistry* **24**, 8094–8101 (1985).
8. Arshavsky, V. Y., Antoch, M. P. & Philippov, P. P. *FEBS Lett.* **224**, 19–22 (1987).
9. Sitarumayya, A. *et al. Biochemistry* **27**, 4880–4887 (1988).
10. Tasaki, I. & Nakaye, T. *Science* **227**, 654–655 (1985).
11. Tasaki, I. & Nakaye, T. *Biophys. J.* **50**, 285–293 (1986).
12. Hagins, W. A. *et al. Proc. Natn. Acad. Sci. U.S.A.* **86**, 1224–1228 (1989).
13. Chabre, M. *et al. Cold Spring Harb. Symp. quant. Biol.* **53**, 313–323 (1988).
14. Vuong, T. M., Chabre, M. & Stryer, L. *Nature* **311**, 659–661 (1984).
15. Wilden, U., Hall, S. W. & Kühn, H. *Proc. natn. Acad. Sci. U.S.A.* **83**, 1174–1178 (1986).
16. Schleicher, A., Kühn, H. & Hofmann, K. P. *Biochemistry* **28**, 1770–1775 (1989).
17. Sitarumayya, A. *Biochemistry* **25**, 5460–5468 (1986).
18. Hofmann, K. P., Emeis, D. & Schmetkamp, P. P. *Biochim. biophys. Acta* **725**, 60–70 (1983).
19. Koch, K.-W. & Stryer, L. *Nature* **334**, 64–66 (1988).
20. Yee, R. & Liebman, P. A. *J. biol. Chem.* **252**, 8902–8909 (1978).
21. Dratz, E. A. *et al. Biochem. biophys. Res. Commun.* **146**, 379–386 (1987).
22. Arshavsky, V. Y. *et al. FEBS Lett.* **250**, 353–356 (1989).
23. Higashijima, T., Ferguson, K. M. & Sternweis, P. J. *J. biol. Chem.* **262**, 3597–3602 (1987).
24. Landis, C. A. *et al. Nature* **340**, 692–696 (1989).
25. Robinson, A. L. *Science* **200**, 1371–1374 (1978).
26. Kühn, H. *Curr. Top. Membranes Transp.* **15**, 171–201 (1981).

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Development of venous occlusions in mice transgenic for the plasminogen activator inhibitor-1 gene

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THE fibrinolytic potential of the vasculature is modulated primarily by the availability and activity of plasminogen activators, which convert the zymogen plasminogen into the active fibrin-degrading enzyme plasmin¹. The activities of these key regulatory enzymes are directly neutralized by their primary endogenous inhibitor, plasminogen activator inhibitor-1 (PAI-1)^{2–6}. Although some individuals with a tendency to develop thrombotic disorders exhibit elevated levels of PAI-1 in their plasma^{7–10}, the cause-and-effect relationship between increased PAI-1 and thrombosis is still unclear. Specifically, it is not known whether chronic depression of fibrinolytic activity results in the development of thrombosis. To address this question we developed transgenic mice in which the contribution of PAI-1 to thrombus formation could be evaluated. The results presented in this report indicate that elevated levels of PAI-1 contribute to the development of venous but not arterial occlusions.

The DNA construct used in the production of PAI-1 transgenic mice contained the murine metallothionein I promoter, human endothelial cell PAI-1 complementary DNA, and the bovine growth hormone polyadenylation signal sequence. Mice putatively transgenic for human PAI-1 showed a single, early indication of disturbance of the haemostatic system (Fig. 1a and b). A distinct area of subcutaneous haemorrhage was

apparent at the tip of the tails of mice at about 3 days after birth. This change became increasingly severe as the mice aged, until by ~12 days after birth the tip of the tail was necrotic and, in addition, the mice had swollen hind feet (Fig. 1c, right). At this age, no other changes were noted. Putative nontransgenic litter mates had normal tails and hind feet (Fig. 1c, left). The necrotic tails and swollen hind feet seemed to be the result of venous occlusions detected histologically (Fig. 1e and f). These occlusions were seen in pups as young as 3 days (the earliest histological examination) and were localized exclusively in the venous circulation; the arteries of the distal tail and limbs were patent. This localization is probably related to the observed haemorrhage and oedema. The thrombi consisted predominantly of mononuclear leukocytes, but also contained platelets, erythrocytes and fibrin. The presence of muscle degeneration and inflammation in 3-day-old mice indicates very early onset of the occlusive disorder, and may at least partially explain why cellular, as opposed to simply fibrin-platelet, thrombi were primarily detected. By ~2 weeks of age, the necrotic portion of the tails had completely sloughed off, whereas the swollen hind limbs appeared normal. This resolution of the occlusions in, and recovery of, the hind limbs of the mice may have been caused by compensatory mechanisms (for example, increased plasminogen activator expression) or by a decline in PAI-1 transgene expression. Therefore, the relationship between the time-dependent existence of the lesions and the levels of PAI-1 DNA, messenger RNA and protein was examined.

DNA extracted from the tails of mice displaying the circulatory disturbances described hybridized with a radiolabelled human PAI-1 cDNA probe, whereas DNA obtained from litter mates having normal tails or from control mice showed little or no hybridization (Fig. 2). Based on these results, 13 founder (G_0) animals were identified, having gene copy numbers of 1–10. In all instances, the existence of the venous occlusions correlated with the presence of the PAI-1 transgene. Assays for PAI-1 protein, activity, and mRNA were then used to correlate PAI-1

TABLE 1 PAI-1 DNA, mRNA and protein in tissues of transgenic mice

Phenotype		Tissue					
(a)		Spleen	Liver	Kidney	Heart	Brain	Lung
Control		ND	6.1	ND	0.8	0.1	0.1
Normal tail		5.8	6.9	8.0	1.1	1.1	0.2
Short tail		438.2	176.3	126.5	12.7	12.2	7.0

(b)		DNA			mRNA			Protein		
Age (days):		4	7	28	4	7	28	4	7	28
Control		0	0	0	<0.5	<0.5	<0.5	10	4	6
Normal tail		4	2	2	1.4	2.5	<0.5	11	10	9
Short tail		11	8	8	5.6	3.5	<0.5	210	98	21

a, Levels of PAI-1 protein in tissues obtained from 4-day-old control and G_1 and G_2 mice of differing phenotypes. Values are expressed as nanograms of PAI-1 per g wet weight of tissue. ND, not detectable. b, PAI-1 DNA copy number and levels of PAI-1 mRNA and protein in samples of liver tissue obtained from control and G_1 and G_2 transgenic mice of various ages. Messenger RNA values are expressed as pg per μ g of total RNA and protein values are expressed as ng per g wet weight of tissue. Three to five animals were analysed in each group. DNA was prepared as described in Fig. 2. The DNA samples were then assayed by Southern blot analysis in which 10 μ g was digested with restriction enzymes, electrophoresed in a 1.0% agarose gel, blotted onto nitrocellulose, and probed with radiolabelled PAI-1 cDNA. PAI-1 gene copy-number of the transgenic mice was determined using standard amounts of the injected transgene in parallel lanes of a Southern blot of the transgenic mouse genomic DNA. RNA was prepared as described in ref. 16 and measured spectrophotometrically. RNA preparations (10 μ g) were denatured in 12 $\times$ SSC and 15% formaldehyde at 60 °C for 15 min. The denatured RNA was applied to nitrocellulose using a slot blot apparatus (Schleicher & Schuell) and baked at 80 °C *in vacuo*. The RNA samples were then probed with radiolabelled PAI-1 cDNA, and PAI-1 mRNA levels were determined by comparison to a PAI-1 mRNA standard curve generated from the PAI-1 cDNA in pBS using the T3 promoter. For PAI-1 protein determinations, tissues were weighed and then extracted in buffer containing 0.1% Triton X-100. After centrifugation of the extracts, the supernatant fluids were analysed for PAI-1 protein using a monoclonal antibody enzyme-linked immunosorbent assay (American Diagnostica).

FIG. 1 Morphology and histological analysis of PAI-1 transgenic mice. *a*, Putative PAI-1 transgenic mouse photographed 3 days after birth. *b*, Higher magnification view of the tail region shown in *a*. *c*, Mice photographed 12 days after birth. Left, mouse with normal tail and hind feet; right, transgenic mouse showing necrosis and sloughing of the tail and swollen hind feet. *d*, Histological cross section through the tail vein of 7-day-old PAI-1 transgenic mouse with necrosis of the tail. Note that the cellular thrombus appears to be surrounding a valve leaflet. *e*, Section through the swollen hind foot of a PAI-1 transgenic mouse. Note that the vein (V) appears to be completely occluded, whereas the artery (A) appears normal.

METHODS. The transgene construct for PAI-1 was assembled as a four-part ligation. A *Bam*HI fragment (1,397 base pairs (bp)) was isolated from a PAI-1 plasmid containing the complete coding region, 80 bases of the 5' untranslated region, and 140 bases of the 3' untranslated sequence (obtained from human umbilical vein endothelial cells). The PAI fragment was ligated on the 5' end to a 1,800-bp *Eco*RI-*Bgl*III metallothionein promoter fragment derived from pDBPV-MMT neo (342-12) (obtained from P. Howley, NIH) and on the 3' end to a 220-bp *Bam*HI-*Sph*I fragment from the polyadenylation signal sequence of bGH (obtained from F. Rottman, Case Western). The vector was pBS (Bluescribe; Stratagene) prepared as an *Eco*RI-*Sph*I fragment. The metallothionein promoter and the bGH polyadenylation signal DNA were phosphorylated and ligated to the PAI DNA overnight 12 °C at a 2:1 molar ratio. In collaboration with DNX (Athens, Ohio) eggs from B6/SJL-F₁J mice were microinjected with the ~3,400-bp *Eco*RI-*Sph*I DNA fragment and implanted in foster mothers according to established protocols. Mice were photographed with a Nikkormat camera using a medical lens and Kodak 100 ASA, daylight-balanced film. For histological evaluation, 30 G₁ and G₂ mice were killed at various ages by CO₂ inhalation. Tissues were fixed by immersion in 10% neutral-buffered formalin, processed, and embedded in paraffin according to established procedures. Tissue cross sections (5 µm) were cut, stained with haematoxylin and eosin, and examined by light microscopy.

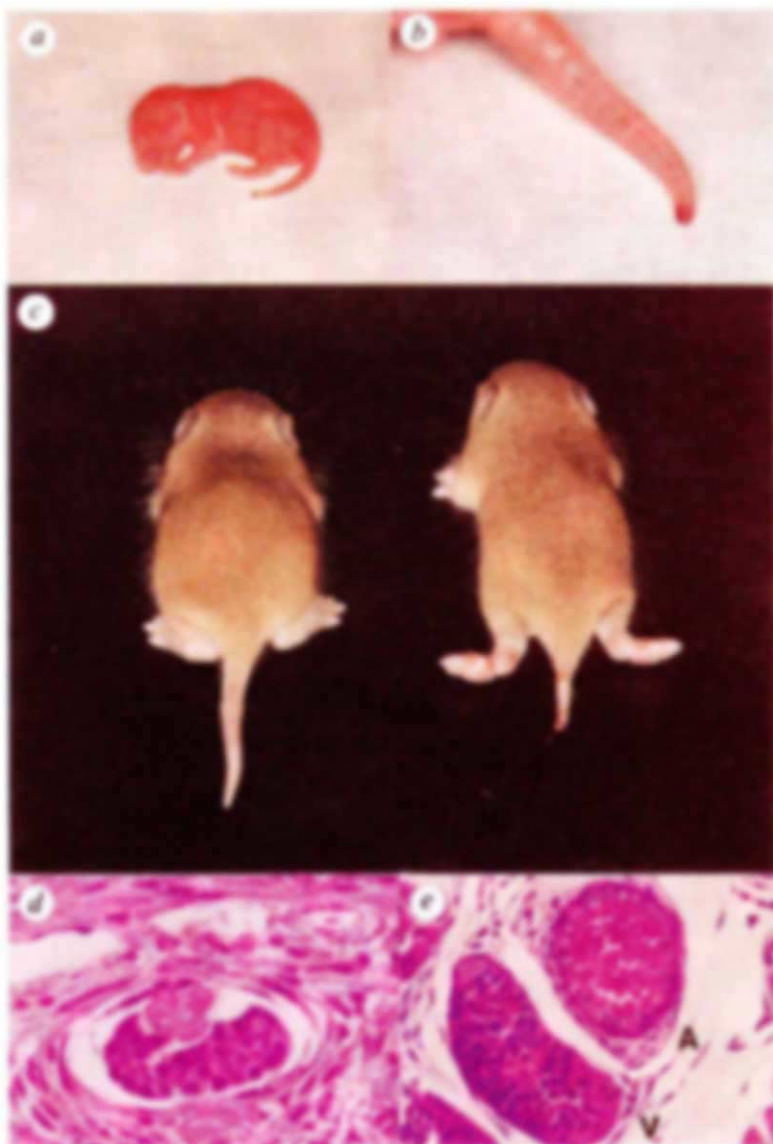
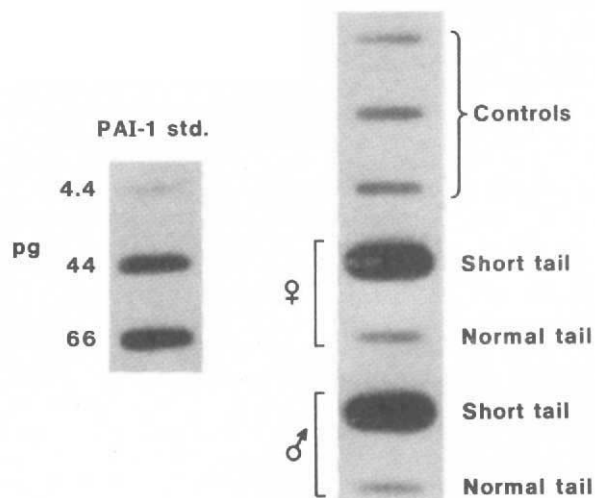


FIG. 2 PAI-1 DNA in tails of adult transgenic mice. Left, PAI DNA standard (std.); top right, tail DNA obtained from control mice; bottom right, tail DNA from female and male short-tailed and normal-tailed mice.

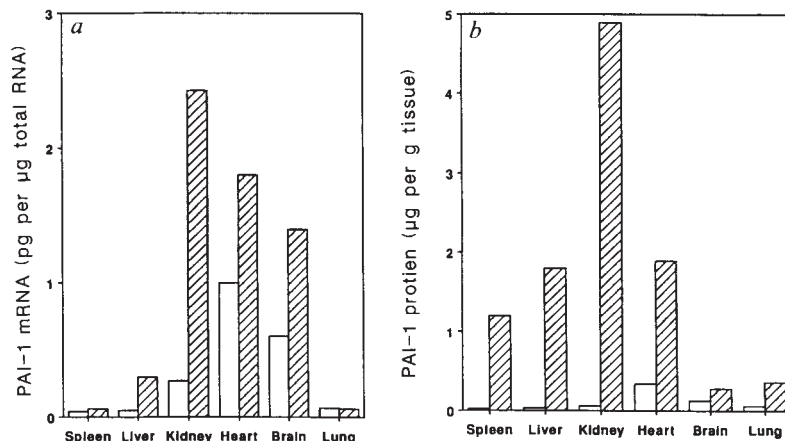
METHODS. DNA was prepared from tails by dicing them with a razor blade into 700 µl of SSTE buffer (100 mM NaCl, 1% SDS, 50 mM Tris buffer and 15 mM EDTA, pH 8.0), then digestion with 0.5 µg ml⁻¹ proteinase K at 55 °C for 12 h. The mixture was then treated with 0.2 µg µl⁻¹ RNase at 37 °C for 1 h. An equal volume of phenol was added and the mixture was extracted twice. The aqueous phase was extracted with chloroform-isoamyl alcohol (24:1) and precipitated with isopropanol. The DNA was pelleted and washed with 70% ethanol and dried under vacuum. Buffer (100 µl TE) was added to each sample and the DNA quantitated spectrophotometrically. For slot blots, 10 µg purified DNA was fixed onto nitrocellulose and probed with a radiolabelled PAI-1 cDNA.



expression with the morphological and histological profiles of the mice. Of the thirteen G₀ mice, six were selected for generation of the G₁ and G₂ lines used for subsequent analyses. Table 1(a) clearly indicates that tissue levels of PAI-1 protein also correlated directly with the presence of occlusions. Presumably, baseline PAI-1 detected in control and normal-tail animals reflects a low level of cross-reactivity between the anti-human PAI-1 monoclonal antibodies and endogenous murine PAI-1. Increased amounts of PAI-1 in the liver and kidney were expected on the basis of previous studies using the metallothionein promoter¹¹; however, the raised level of PAI-1 protein detected in spleen tissue obtained from transgenic animals was surprising,

and is as yet unexplained. The PAI-1 content of the cellular occlusions was not examined. These data show a strong link between the existence of venous occlusions and the presence and expression of the PAI-1 transgene. This relationship was examined more closely by determining the PAI-1 DNA copy-number and by measuring the PAI-1 mRNA and protein levels

FIG. 3 Induction of PAI-1 expression in 28-day-old transgenic mice with ZnSO_4 . *a*, PAI-1 mRNA in tissues of transgenic mice. *b*, PAI-1 protein in tissues of transgenic mice. Open bars, PBS control; hatched bars, ZnSO_4 -injected. PBS or PBS containing ZnSO_4 was administered to 28-day-old mice IP (10 mg kg^{-1}). Eight hours after injection, mice were killed by cervical dislocation and tissues removed for RNA and protein analyses as described in Table 1.



in the livers of mice killed at different times after birth (Table 1(b)). As expected, DNA copy-number did not vary significantly within a phenotype. Both PAI-1 mRNA and protein declined, however, in the transgenic ('short tail') mice 4–28 days after birth to levels detected in control and normal-tail (non-expressing transgenic) mice. This decline paralleled the resolution of the venous occlusions in the hind limbs, and provides additional evidence of a strict correlation between the presence and expression of the PAI-1 transgene and the development of occlusions.

The decline of PAI-1 expression during the first 4 weeks after birth could result from the loss of responsiveness of the metallothionein promoter to endogenous control mechanisms. We addressed this issue by administering ZnSO_4 to 28-day-old transgenic mice that had shown tail necrosis and swollen hind limbs earlier in life. This treatment induced the expression of the PAI-1 gene from baseline levels to levels that in certain tissues exceeded those observed at day 4 (Fig. 3). Plasma PAI-1 was increased by ~20-fold after ZnSO_4 treatment (data not shown). We conclude from these experiments that the metallothionein promoter was intact and responsive. Studies of the mouse¹² and the chick¹³ have shown that expression of endogenous metallothionein peaks shortly before birth (mouse) or after birth (chick) and declines thereafter. Taken together, these data indicate that transgenic PAI-1 may decline after day 4 as a consequence of the normal function of mechanisms governing the expression of endogenous metallothionein.

We found that the level of PAI-1 protein directly reflects the amount of PA neutralizing activity detectable (data not shown). In addition, the occlusions in the PAI-1 transgenic mice are reminiscent of those present in protein C-deficient patients in that they are localized to the venous side of the circulation¹⁴. Thus, it is conceivable that neutralization of endogenous murine activated protein C may also contribute to the observed venous occlusive disorder. Distinguishing between these and other possibilities is made particularly difficult by the fact that the critical period of evaluation is before 4 days after birth. Indeed, it has not been possible to measure the endogenous circulating levels of these murine pro-fibrinolytic factors because of the small amounts of blood obtainable from mice of this age. Alternatively, the occlusions may result from a perturbation of the vascular endothelium that is associated with chronically elevated PAI-1. In this regard, PAI-1 binds to and is protected from inactivation by vitronectin, an important component of the extracellular matrix¹⁵. The cellular nature of the venous occlusions suggests that such a perturbation may have affected the cellular adhesive properties of the venous endothelium.

Regardless of the precise mechanism, however, the results presented here provide strong evidence that elevated PAI-1 is closely associated with the development of venous occlusions. This finding has important implications in the development of

approaches to predict, treat, and possibly prevent thrombotic disorders by monitoring and regulating the endogenous fibrinolytic system. □

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- Gaffney, P. J. *Haemostasis and Thrombosis* (eds Bloom, A. L. & Thomas, D. P.) 198–224 (Churchill Livingstone, Edinburgh, 1981).
- Schleef, R. R. & Loskutoff, D. J. *Haemostasis* **18**, 328–341 (1988).
- Loskutoff, D. J. *Sem. Thromb. Haemostasis* **14**, 100–109 (1988).
- Erickson, L. A., Ginsberg, M. H. & Loskutoff, D. J. *J. clin. Invest.* **74**, 1465–1472 (1984).
- van Mourik, J. A., Lawrence, D. A. & Loskutoff, D. J. *J. biol. Chem.* **259**, 14914–14921 (1984).
- Kruijthof, E. K. O., Tran-Thang, C. & Bachmann, F. *Blood* **70**, 1645–1653 (1987).
- Nilsson, I. M., Ljunggren, H. & Tengborn, L. *Br. med. J.* **290**, 1453–1456 (1985).
- Paramo, J. A., Alfaro, M. J. & Rocha, E. *Thromb. Haemostasis* **54**, 713–716 (1985).
- Hamsten, A., Wiman, B., deFaire, U. & Blomback, M. *New Engl. J. Med.* **313**, 1557–1563 (1985).
- Hamsten, A. *et al. Lancet* **2**, 3–9 (1987).
- Durnam, D. M. & Palmiter, R. D. *J. biol. Chem.* **256**, 5712–5716 (1981).
- Quaife, C., Hammer, R. E., Mottet, N. K. & Palmiter, R. D. *Dev. Biol.* **118**, 549–555 (1986).
- Chakraborty, T. & Biswas, B. B. *Biochem. biophys. Res. Commun.* **147**, 226–233 (1987).
- Horellou, M. H., Conard, J., Bertina, R. M. & Samama, M. *Br. med. J.* **289**, 1285–1287 (1984).
- Declercq, P. J. *et al. J. biol. Chem.* **263**, 15454–15461 (1988).
- Chomczynski, P. & Sacchi, N. *Analyt. Biochem.* **162**, 156–159 (1987).

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Drosophila Krüppel protein is a transcriptional repressor

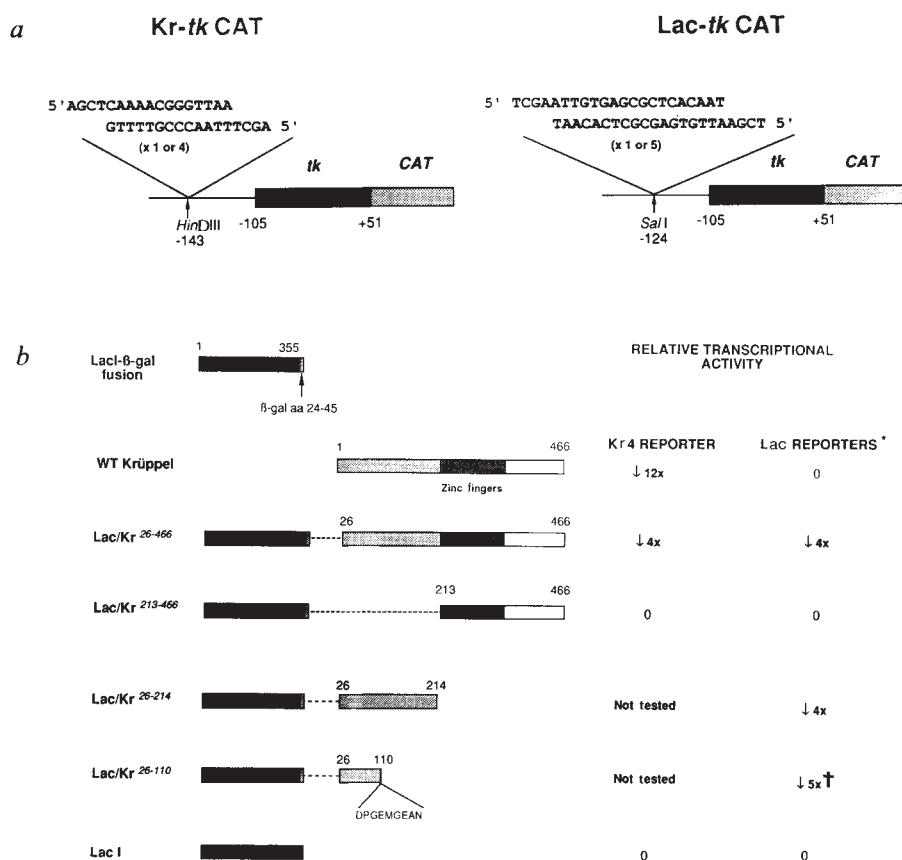
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KRÜPPEL (Kr), one of the zygotically active *Drosophila* segmentation genes, is expressed in a restricted domain during the blastoderm stage of embryogenesis and is involved in the control of development of the thoracic and abdominal segments of the fly¹. Kr encodes a polypeptide containing DNA-binding zinc-finger motifs², disruptions of which yield Kr mutants^{3,4}. We have assayed the transcriptional activities of wild-type Kr protein as well as Lac repressor/Kr fusion proteins in HeLa and CV-1 cells. Wild-type Kr and a Lac-Kr chimaeric protein repressed transcription from reporter promoters in which a consensus Kr binding site derived from sequences within the *even-skipped* promoter⁵ had been inserted in an upstream position. We mapped the repression function of Kr to an alanine-rich amino-terminal region of the protein, as a Lac/Kr fusion protein containing only amino acids 26–110 of Kr repressed transcription from a reporter promoter containing

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FIG. 1 a, Reporter constructs containing either synthetic duplex oligonucleotide Krüppel binding sites or *lac* operator sequences upstream of the HSV *tk* promoter. b, The structure of wild-type (WT) Kr and Lac-Kr chimaeric proteins. The table on the right of the figure indicates the relative transcriptional activities of Kr and Lac/Kr chimaeric proteins on the Lac-tkCAT and Kr4-tkCAT reporters as determined from 2–4 independent experiments. *Lac5-tkCAT was used in the demonstration of repression by Lac/Kr^{26–110}, whereas Lac1-tkCAT was the reporter gene in all other experiments. No significant difference in fold repression was noted when Lac/Kr^{26–214} was co-expressed with Lac5-tkCAT versus Lac1-tkCAT. †Activities were assayed from transfection into HeLa cells, except for experiments with Lac/Kr^{26–110}, which were performed in CV-1 cells. METHODS. Kr1-tkCAT and Kr4-tkCAT were constructed by the insertion of one or four duplex oligonucleotide Kr-binding sites⁵, respectively, into the unique *Hind*III site of pBLCAT2 (ref. 21), which contains HSV *tk* sequences from –105 to +51. Lac1-tkCAT and Lac5-tkCAT were constructed by inserting one or five copies of a symmetrical *lac* operator sequence²² with high affinity for lacI into the *Sal*I site of pBLCAT2. The pBLCAT2 plasmid also contains a wild-type *lac* operator located downstream of the *CAT* gene, within the pUC18 backbone, with an eightfold lower affinity for lacI. The lac repressor was expressed from plasmid pCMVlacI (ref. 22), containing the simian cytomegalovirus promoter. Wild-type Kr was expressed from a construct in which *lacI* sequences in pCMVlacI were replaced with the 2.3-kilobase (kb) *Eco*RI insert of pCK2B (ref. 2). The Lac/Kr fusion genes were constructed by linking a 1.4-kb *Bgl*II–*Pvu*I fragment encoding lac repressor amino acids 1–355 linked to 23 N-terminal amino acids (aa) of β -galactosidase²³ to portions of the Kr cDNA using synthetic duplex oligonucleotide adaptors to maintain the proper reading frame. The adaptors insert two additional amino acids (leucine and glutamic acid) between *lac* and Kr sequences. These fusions were cloned into Bluescript SK+ (Stratagene), excised by digestion with *Sac*I and *Eco*RI, then cloned into pCMVlacI digested with *Sac*I and *Eco*RI. The Lac/Kr^{26–214} construct was made by restriction of Lac/Kr^{26–466} at a unique *Bam*HI site at nucleotide 769 of the Kr cDNA, followed by end-repair with Klenow fragment and ligation to a linker contain-



ing stop codons in all three reading frames²⁴. Lac/Kr^{26–110} was constructed by *Bal*/31 digestion of Lac/Kr^{26–466}, followed by ligation to HSV-*tk* polyadenylation sequences²⁵. Nine amino acids (DPGEMGEAN; single-letter code) were added to the Kr amino-acid sequence by its fusion to the HSV-*tk* sequence through a *Bam*HI linker added to the *Sma*I site at nucleotide, 1,417 of *tk*, preceding the termination codon. Constructs were confirmed by digestion of plasmids with restriction enzymes and by dideoxynucleotide sequencing of plasmids.

upstream *lac* operators. This demonstrates that the DNA-binding and repression activities of the Kr protein are distinct. These data are consistent with genetic evidence that Kr represses *even-skipped*^{6,7} and *hunchback*⁸ expression, and suggest that Kr is a negative regulator of transcription in *Drosophila*.

Mutations in *Kr* affect the expression of other gap genes^{8,9} as well as pair-rule and homeotic genes^{6,7,10,11}. The *Kr* gene product has been identified as a putative transcription factor by virtue of its zinc-finger motifs², nuclear location¹², and ability to bind specifically to DNA^{5,9,13}. To test for transcriptional activity of the *Kr* gene product, the *Kr* complementary DNA was expressed in human and monkey cells from an expression plasmid containing the simian cytomegalovirus (CMV) promoter. This plasmid was co-transfected with the herpes simplex virus thymidine kinase (HSV-*tk*) promoter, containing one or four *Kr*-binding sites at nucleotide –143 (Kr1-tkCAT and Kr4-tkCAT, respectively) linked to a reporter gene (chloramphenicol acetyltransferase, CAT) (Fig. 1a). Expression of *Kr* protein led to a fourfold decrease in CAT activity from Kr1-tkCAT, and a 17-fold decrease in CAT activity from Kr4-tkCAT (Fig. 2a), compared with controls of the same reporter constructs co-transfected with a plasmid expressing the lac repressor from the CMV promoter. Cotransfection of the *Kr* cDNA with a reporter construct lacking *Kr* binding sites did not significantly affect CAT expression (Fig. 2a). To localize the region(s) in the *Kr* protein responsible for repressing transcription from the CAT

reporter genes, fusions between sequences encoding portions of the *Kr* protein and of a heterologous DNA-binding protein, the lac repressor (lacI), were constructed (Fig. 1b). A fusion between lacI and amino-acid residues 26–466 of *Kr* (Lac/Kr^{26–466}) also repressed CAT expression from Kr4-tkCAT, whereas a fusion between lacI and residues 213–466 (Lac/Kr^{213–466}) of *Kr* did not (Fig. 2b). In addition, Lac/Kr^{26–466} repressed CAT expression from a reporter construct containing an upstream *lac* operator at nucleotide –124 (Lac1-tkCAT), whereas a fusion lacking the N-terminal portion of *Kr* did not (Fig. 2c). The sequence encoding the N-terminal portion of *Kr* alone, deleting the zinc-finger domain required for DNA-binding activity (see below), was fused to *lacI* by insertion of a translation termination linker after residue 214 of *Kr* (Lac/Kr^{26–214}). Lac/Kr^{26–214}, like Lac/Kr^{26–466}, repressed transcription from Lac1-tkCAT (Fig. 2c). This demonstrates that the DNA-binding and repression functions of *Kr* are distinct. To further localize the region of *Kr* responsible for repression, Lac/Kr^{26–214} and Lac/Kr^{26–110} were each transfected along with a reporter construct containing five *lac* operators (Lac5-tkCAT) (Fig. 2d). The two fusion proteins repressed CAT expression to similar levels, indicating that an 85-amino acid region of *Kr* encodes the transcriptional repression activity. None of the Lac/Kr chimaeric constructs repressed CAT expression from a reporter devoid of *Kr* binding sites or high-affinity upstream *lac* operators (data not shown). Results from all transfections are summarized in Fig. 1b.

(Fig. 1b). This emphasizes the separate nature of the DNA-binding and repression activities of Kr.

The ability of Kr to repress transcription of the reporter constructs described above is consistent with the role of Kr in limiting the boundaries of expression of the gap gene *hunchback* (*hb*)⁸ and the pair-rule gene *even-skipped* (*eve*)^{6,7} during the blastoderm stage of *Drosophila* development. Our data demonstrate that the Kr protein functions across species lines and contains a transcriptional repression activity, distinct from its DNA-binding activity, that can act at a distance from the start site of transcription. Repression is unlikely to result from steric hindrance, as the Kr and lacI binding sites were placed relatively far from the upstream activator-binding sites of the HSV-*tk* promoter and the start site of HSV-*tk* transcription¹⁴. Kr repression of the *eve* promoter might occur by competitive binding between Kr and the *hb* protein (Hb) to adjacent binding sites in the *eve* promoter, leading to the displacement of Hb, an activator of the *eve* promoter. Kr bound immediately adjacent to Hb might also mask or quench Hb activity by a protein-protein interaction⁵. Our data are more consistent with a direct model for repression at a distance¹⁵, in which the N-terminal region of Kr would interact with more proximal components of the transcription machinery, such as upstream activator proteins (for example, CTE, TFIID), RNA polymerase II or its accessory proteins, thereby blocking the usual stimulation of transcription.

Comparison of the N-terminal region of Kr between amino acids 26 and 110 with the amino-acid sequence of two other *D. melanogaster* transcriptional repressors, *engrailed*^{16,17} and *even-skipped*^{17,18}, reveals the existence of similar alanine-rich subsegments (Fig. 3). In addition, one region within the *engrailed* protein that is similar to Kr is evolutionarily conserved between *D. melanogaster* and *D. virilis*¹⁹. Although the functional significance of these alignments is not known, we are analysing deletions, point mutations and exchanges of sequences among these proteins to determine whether the aligned regions encode a common transcriptional repression activity. Such regions could mediate protein-protein interactions necessary to sequester components of the transcriptional machinery from interactions required to initiate transcription.

Genetic data indicate that Kr also acts as an activator of expression of the gap gene *knirps*, the promoter of which has been shown to contain Kr-binding sites⁹. This activation may depend on the context of the *knirps* promoter, and could be encoded by a different region of Kr. The *Ultrabithorax* (*Ubx*) protein both activates the *Ubx* promoter and represses the *Antennapedia* promoter; however, the activation function of the protein can be deleted without affecting repression activity²⁰. We note that a C-terminal deletion of Kr after amino acid 377 yields a hypomorphic Kr phenotype⁴, yet this region is not required for the repression function of Kr in our assay system.

Note added in proof: Deletion of the alanine-rich region of *eve* that can be aligned with Kr impairs the ability of *eve* to function as a transcriptional repressor in cultured *Drosophila* cells (K. Han and J. L. Manley, personal communication). □

16. Jaynes, J. B. & O'Farrell, P. H. *Nature* **336**, 744–749 (1988).
17. Han, K., Levine, M. S. & Manley, J. L. *Cell* **56**, 573–583 (1989).
18. Biggin, M. A. & Tjian, R. *Cell* **58**, 433–440 (1989).
19. Kassiss, J. A., Poole, S. J., Wright, D. K. & O'Farrell, P. H. *EMBO J.* **5**, 3583–3589 (1986).
20. Krasnow, M. A., Saffman, E. E., Kornfeld, K. & Hogness, D. S. *Cell* **57**, 1031–1043 (1989).
21. Luikow, B. & Schütz, G. *Nucleic Acids Res.* **15**, 5490 (1987).
22. Figge, J., Wright, C., Collins, C. J., Roberts, T. M. & Livingston, D. M. *Cell* **52**, 713–722 (1988).
23. Brake, A. J., Fowler, A. V., Zabin, I. Z., Kania, J. & Müller-Hill, B. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4824–4827 (1978).
24. DeLuca, N. A. & Schaffer, P. A. *Nucleic Acids Res.* **15**, 4491–4511 (1987).
25. McKnight, S. L. *Nucleic Acids Res.* **8**, 5949–5964 (1980).
26. Selden, R. F., Howie, K. B., Rowe, M. E., Goodman, H. M. & Moore, D. D. *Molec. cell Biol.* **6**, 3173–3179 (1986).
27. Gorman, C. M., Moffat, L. F. & Howard, B. H. *Molec. cell Biol.* **2**, 1044–1051 (1982).
28. Smith, T. F. & Waterman, M. S. *J. molec. Biol.* **147**, 195–197 (1981).
29. Ralph, W. W., Webster, T. & Smith, T. F. *Comput. Appl. Biol. Sci.* **3**, 211–216 (1987).

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Rapid evolution in response to high-temperature selection

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TEMPERATURE is an important environmental factor affecting all organisms^{1,2}, and there is ample evidence from comparative physiology that species^{1,3} and even conspecific populations^{4,5} can adapt genetically to different temperature regimes. But the effect of these adaptations on fitness and the rapidity of their evolution is unknown, as is the extent to which they depend on pre-existing genetic variation rather than new mutations. We have begun a study of the evolutionary adaptation of *Escherichia coli* to different temperature regimes, taking advantage of the large population sizes and short generation times in experiments on this bacterial species^{6–10}. We report significant improvement in temperature-specific fitness of lines maintained at 42 °C for 200 generations (about one month). These changes in fitness are due to selection on *de novo* mutations and show that some biological systems can evolve rapidly in response to changes in environmental factors such as temperature.

A clone of a single cell in the ancestral line maintained at 37 °C for 2,000 generations was used to found six high-temperature (42 °C) and six control (37 °C) lines. All lines (ancestral, high-temperature and control) were propagated in Davis minimal medium¹¹ supplemented with 25 µg ml⁻¹ glucose. Cultures (10 ml) were maintained by daily serial dilution into fresh medium, permitting a 100-fold increment in biomass (log₂ 100 = 6.64 generations of binary fission) each day. The total size of a population maintained under this culture regime cycles from ~5 × 10⁶ to ~5 × 10⁸ cells. The founding lines possessed one of two arabinose-utilization marker states^{9,10}, which were used for clonal identification in fitness assays and for monitoring possible cross-contamination between lines. Selective neutrality of these markers at both 37 °C and 42 °C was verified by preliminary experiments and no cross-contamination of lines was observed. Additional phenotypic markers were also used to exclude the possibility of contamination from external sources^{9,10}. The use of an ancestral line that was already well-adapted to the culture medium increased the likelihood that temperature-specific adaptations could be detected, as opposed to other beneficial but non-temperature-specific mutations that might enhance adaptation to the culture medium. Also, by studying spontaneous mutants that have increased in frequency gradually by selection rather than mutants obtained by mutagenesis and subsequent screening for extreme phenotypes, one avoids mutations that have profoundly deleterious pleiotropic effects and consequently would have little evolutionary significance.

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1. Gaul, U. & Jäckle, H. *Trends Gen.* **3**, 127–131 (1987).
2. Rosenblatt, U. B. *et al. Nature* **319**, 336–339 (1986).
3. Redemann, N., Gaul, U. & Jäckle, H. *Nature* **332**, 90–92 (1988).
4. Gaul, U., Redemann, N. & Jäckle, H. *Proc. natn. Acad. Sci. U.S.A.* **86**, 4599–4603 (1989).
5. Stanojević, D., Hoey, T. & Levine, M. *Nature* **341**, 331–335 (1989).
6. Frasch, M. & Levine, M. *Genes Dev.* **1**, 981–995 (1987).
7. Goto, T., Macdonald, P. & Maniatis, T. *Cell* **57**, 413–422 (1989).
8. Jäckle, H., Tautz, D., Schuh, R., Seifert, E. & Lehmann, R. *Nature* **324**, 668–670 (1986).
9. Pankratz, M. J., Hoch, M., Seifert, E. & Jäckle, H. *Nature* **341**, 337–339 (1989).
10. Ingham, P. W., Ish-Horowitz, D. & Howard, K. R. *EMBO J.* **5**, 1659–1665 (1986).
11. Harding, K. & Levine, M. *EMBO J.* **7**, 205–214 (1988).
12. Gaul, U., Seifert, E., Schuh, R. & Jäckle, H. *Cell* **50**, 639–647 (1987).
13. Treisman, J. & Desplan, C. *Nature* **341**, 335–337 (1989).
14. McKnight, S. L. *Cell* **31**, 355–365 (1982).
15. Levine, M. & Manley, J. L. *Cell* **59**, 405–408 (1989).

TABLE 1 Improvements in fitness during 400 generations at 42 °C

Generation	Estimated mean fitness over all lines*	Estimated variance in fitness among lines†
100	$\bar{X}=0.991$ $t_s=-1.386$ $0.5 < P < 0.9$	$s_g^2=2.07 \times 10^{-6}$ $F_s=1.008$ $0.25 < P < 0.5$
200	$\bar{X}=1.085$ $t_s=2.712$ $0.01 < P < 0.025$	$s_g^2=4.88 \times 10^{-3}$ $F_s=6.122$ $P < 0.001$
400	$\bar{X}=1.072$ $t_s=2.827$ $0.01 < P < 0.025$	$s_g^2=2.92 \times 10^{-3}$ $F_s=4.082$ $0.001 < P < 0.01$

* The mean and standard error of the mean were calculated from the means of the six fitness assays performed for each of the six lines. The corresponding *t*-statistic is shown, as is the significance level associated with the one-tailed hypothesis that the mean fitness across all lines relative to the ancestral clone was greater than one. The number of degrees of freedom employed in this test was five, corresponding to one less than the number of lines.

† Variance component due to differences in fitness among lines¹⁴. The corresponding *F*-statistic is shown, along with the significance level associated with the hypothesis that this variance was greater than zero. The corresponding degrees of freedom for the numerator and denominator in the *F*-ratio were 5 and 30, respectively.

After 100, 200 and 400 generations of binary fission (15, 30 and 60 days respectively), single colony isolates from each high-temperature line were made to compete against the ancestral clone possessing the alternative arabinose marker state. These competition experiments used the same culture conditions in which the high-temperature lines had been maintained. Six replicate competition experiments were run for each of the six lines at each sample time. Both competitors were preincubated at 42 °C for one day, such that each had equal opportunity to acclimatize physiologically to the culture conditions. Thereafter equal aliquots of each competitor were added to fresh medium; initial densities were determined by plating on medium that permitted the competitors to be distinguished on the basis of the arabinose-utilization marker state. The mixed culture was maintained at 42 °C for one day and the final densities of ancestral and high-temperature clones determined again by plating. These competition experiments enabled us to examine adaptive evolution (that is, improvement in fitness relative to the ancestral clone) by the high-temperature lines. Fitness (*W*) was defined as the ratio of the number of doublings for the high-temperature and ancestral clones: $W = \log_2(N_f/N_i) / \log_2(N_f/N_i)$ where N_i and N_f are the initial densities and N_f and N_i are the final densities of the ancestral and high-temperature clones, respectively^{9,10}. A value of 1 indicates that two clones have equal fitness; a value greater than 1 indicates that the high-temperature line has adapted evolutionarily.

The major results are given in Table 1. At 100 generations of culture at 42 °C there was no significant increase in fitness nor significant heterogeneity in fitness among the six lines. At 200 generations, and again at 400 generations, fitness had increased significantly relative to the ancestral clone by an average of ~7–8%. Furthermore, analyses of variance indicated significant heterogeneity in fitness among the six lines at 200 generations and again at 400 generations. Thus, new mutations that increased fitness at 42 °C had occurred and reached high frequency within only 200 generations (30 days) in at least some of the high-temperature lines.

The control lines provide evidence that the adaptive evolution observed in the high-temperature lines was temperature-specific (Table 2). The six control lines maintained at 37 °C for 400 generations did not show any significant fitness improvement when competed at 37 °C against the same ancestral clone. Relative to their common ancestor, the fitness at 37 °C of the control

lines was significantly less than the fitness at 42 °C of the high-temperature lines (one-tailed *t*-test; $t_s=2.515$, 10 d.f., $0.01 < P < 0.025$).

We also determined whether the adaptive evolution observed in the high-temperature lines produced any correlated response in fitness at 37 °C, the ancestral temperature regime (Table 2). A significant fitness advantage was observed when the high-temperature lines were competed against the ancestor at 37 °C. Although the fitness advantage observed at 37 °C (~4%) was less than the advantage observed at 42 °C (~7%), this difference was not statistically significant (one-tailed *t*-test for paired comparisons, $t_s=0.898$, 5 d.f., $0.1 < p < 0.25$). Also, mean fitnesses at 37 °C and 42 °C were not positively correlated across the high-temperature lines (coefficient of correlation $r=-0.088$, 4 d.f., $P > 0.5$). One possible explanation for this lack of correlation across lines is heterogeneity among alleles in their effects on a correlated character^{9,12}. To examine this possibility we first computed the overall ratio of the correlated response to selection (mean change in fitness at 37 °C, Table 2) to the direct response to selection (mean change in fitness at 42 °C, Table 1): $(1.040-1)/(1.072-1)=0.554$. This ratio provides an estimate of the regression of fitness at 37 °C on fitness at 42 °C (see equation 19.5a in ref. 13). We then used the jackknife procedure¹⁴ to determine the influence of each fitness assay on this ratio. An analysis of variance using the jackknifed data (Table 3) indicates significant heterogeneity among the lines in this ratio, and hence in the relationship between fitnesses at 37 °C and 42 °C. Five of the six lines give a positive regression coefficient (mean slope from jackknifed data = 0.782) and among these five lines there is a significant positive correlation between mean fitnesses at 37 °C and 42 °C ($r=0.968$, 3 d.f., $P < 0.01$). The other line gives a negative regression coefficient (mean slope from jackknifed data = -0.738) that obscures the overall correlation. There is evidently heterogeneity among alleles that improve fitness at 42 °C in their effect on fitness at 37 °C.

Even with such heterogeneity, the average correlated response of fitness at 37 °C for the high-temperature lines was significantly greater than the average direct response of the control lines at 37 °C (two-tailed *t*-test, $t_s=2.982$, 10 d.f., $0.01 < P < 0.025$). This unexpected result demonstrates that adaptation to an environment may sometimes occur more rapidly as a consequence of selection in another environment than as a direct evolutionary response. We see three possible explanations for this phenomenon in our experimental system. First, certain mutations may occur only at higher temperatures. Second, the high-temperature regime may have increased the rate of certain mutations^{6,15}, including some beneficial mutations sufficiently rare that they were not yet observed at 37 °C. Third, equivalent mutations may have occurred at the same rate at 37 °C, but were not fixed during this period because of a smaller selective

TABLE 2 Temperature-specificity of improvements in fitness

Estimated mean fitness over all lines*	Estimated variance in fitness among lines†
Direct response of fitness at 37 °C after propagation at 37 °C for 400 generations	
$\bar{X}=1.006$ $t_s=1.164$ $0.1 < P < 0.25$	$s_g^2=-1.28 \times 10^{-4}$ $F_s=0.587$ $0.5 < P < 0.75$
Correlated response of fitness at 37 °C after propagation at 42 °C for 400 generations	
$\bar{X}=1.040$ $t_s=4.056$ $0.001 < P < 0.01$	$s_g^2=2.13 \times 10^{-4}$ $F_s=1.585$ $0.1 < P < 0.25$

* See Table 1.

† See Table 1.

TABLE 3 Heterogeneity in ratio of correlated response (change in fitness at 37 °C) to direct response (change in fitness at 42 °C) among lines propagated at 42 °C for 400 generations*

Source	Degrees of freedom	Sums of squares	Mean square	F_s
Among lines	5	24.24	4.85	2.76
Within lines	66	115.91	1.76	$0.025 < P < 0.05$
Total	71	140.14		

* The overall ratio of the correlated response (Table 2) to the direct response (Table 1) is calculated in the text. The jackknife procedure¹⁴ was used to compute pseudovalues that indicate the influence of each of the 72 fitness assays (six assays at each of two temperatures for each of six lines) on this overall ratio. The grand mean of the pseudovalues was 0.529, close to the overall ratio. The analysis of variance tests for heterogeneity among lines in pseudovalues. Significant heterogeneity indicates genetic variation among lines in the effects of alleles that improve fitness at 42 °C on fitness at 37 °C. See text for details.

advantage at this temperature (smaller selective advantage both increases the probability that a favourable mutation will be lost initially owing to genetic drift and increases the amount of time that would be required for the favoured mutant allele, if it is not lost by drift, to reach some specified high frequency^{16,17}). These results also bear on the issue of whether there necessarily exists a negative genetic correlation (that is, pleiotropic trade-off) between fitnesses measured in different environments (for example, temperatures¹⁸). The correlated improvement in fitness at 37 °C in most of the high-temperature lines clearly indicates no necessary loss of fitness in the ancestral environment (37 °C) during adaptation to a new environment (42 °C).

Selection studies, in which one monitors the response of laboratory populations to variation in natural environmental variables, may provide a powerful tool to investigate and predict the response of natural populations to environmental variation¹⁹ (for example, climate change^{20,21}). Our experiments demonstrate that some bacteria can respond to changes in temperature in ways that improve evolutionary fitness over short absolute time periods. Mutations favouring such fitness improvement are evidently not rare and can spread rapidly through a population. Some organisms may therefore be able to track and evolve in response to temperature changes in their environments even seasonally and certainly over longer time periods. □

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- Hochachka, P. W. & Somero, G. N. *Biochemical Adaptation* (Princeton University Press, Princeton, New Jersey, 1984).
- Cossins, A. R. & Bowler, K. *Temperature Biology of Animals* (Chapman and Hall, London, 1987).
- Prosser, C. L. *Adaptational Biology: Molecules to Organisms* (Wiley, New York, 1986).
- Powers, D. A. in *New Directions in Ecological Physiology* (eds Feder, M. E., Bennett, A. F., Burggren, W. W. & Huey, R. B.) 102–130 (Cambridge University Press, New York, 1987).
- Watt, W. B. *Am. Nat.* **125**, 118–143 (1985).
- Novick, A. & Szilard, L. *Proc. natn. Acad. Sci. U.S.A.* **36**, 708–719 (1950).
- Atwood, K. C., Schneider, L. K. & Ryan, F. J. *Proc. natn. Acad. Sci. U.S.A.* **37**, 146–155 (1951).
- Dykhuizen, D. E. & Hartl, D. L. *Microbiol. Rev.* **47**, 150–168 (1983).
- Lenski, R. E. *Evolution* **42**, 425–432 (1988).
- Lenski, R. E. *Evolution* **42**, 433–440 (1988).
- Carlton, B. C. & Brown, B. J. in *Manual for Methods for General Microbiology* (ed. Gerhardt, P.) 222–242 (Am. Soc. Microbiol., Washington DC, 1981).
- Cohan, F. M. & Hoffmann, A. A. *Genetics* **114**, 145–163 (1986).
- Falconer, D. S. *Introduction to Quantitative Genetics* (Longman, Essex, 1981).
- Sokal, R. R. & Rohlf, F. J. *Biometry* (Freeman, San Francisco, 1981).
- Ryan, F. J. & Kiritani, K. *J. gen. Microbiol.* **20**, 644–653 (1959).
- Maynard Smith, J. *J. theor. Biol.* **30**, 319–335 (1971).
- Moran, P. A. P. *The Statistical Processes of Evolutionary Theory* (Oxford University Press, Oxford, 1962).
- Huey, R. B. & Hertz, P. E. *Evolution* **38**, 441–444 (1984).
- Huey, R. B. & Kingsolver, J. G. *Trends Ecol. Evol.* **4**, 131–135 (1989).
- National Research Council, Carbon Dioxide Assessment Committee. *Changing Climate* (National Academy, Washington DC, 1983).
- Abrahamson, D. E. *The Challenge of Global Warming* (Island, Washington DC, 1989).

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Proximity to the promoter inhibits recognition of cauliflower mosaic virus polyadenylation signal

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THE replication of retroviruses and pararetroviruses (such as caulimoviruses and hepadnaviruses)¹ involves the production of terminally redundant genomic-length RNA (Fig. 1). The sequence repeated at both ends of the RNA (the R region) contains a polyadenylation signal, and for production of full-length RNA the version of this at the 5' end of the template must be bypassed by RNA polymerase, but the version at the 3' end must be recognized^{2–5}. This implies that the position of the polyadenylation signal determines its efficiency, and we report here experiments aimed at investigating the basis of this phenomenon. Our results with cauliflower mosaic virus⁶ suggest that proximity to the transcription initiation site inhibits messenger RNA 3'-end processing directed by polyadenylation signals.

To test whether the cauliflower mosaic virus (CaMV) R region without additional upstream CaMV sequences was sufficient to direct 3'-end processing in plants, we inserted the chloramphenicol acetyltransferase (CAT) reporter gene at the beginning of the R region (plasmid R-CAT, Fig. 2a). A DNA fragment from the nopaline synthetase gene (*nos*), containing a second polyadenylation signal^{7,8}, was placed downstream of R to rescue RNA molecules that were not processed at the CaMV site. Total RNA was purified from transiently transfected plant protoplasts (*Nicotiana plumbaginifolia*) and analysed in RNase protection assays using an antisense probe (P1) that covered both polyadenylation signals (Fig. 2a, b). Only one major band was obtained, which was of the expected size (190 nucleotides) for a transcript processed at the CaMV site. Processing at this site

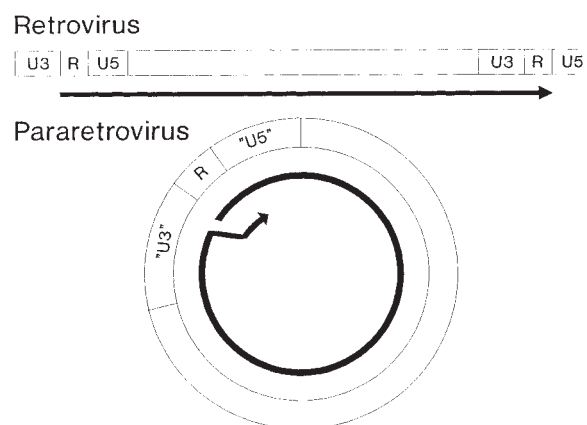


FIG. 1 Production of a terminally redundant genomic RNA in retroviruses and pararetroviruses. Both retroviruses and pararetroviruses¹ (caulimoviruses and hepadnaviruses) reverse-transcribe their genomic RNA but pararetroviruses do not necessarily integrate. In retroviruses, the template for transcription is a provirus integrated into the host-cell genome. The long terminal repeats (LTR) consist of: U3, the unique region at the 3' end of the genomic RNA containing the promoter region; R, the RNA terminal redundancy containing at least part of the polyadenylation signal; and U5, the unique region at the 5' end of the RNA. In cauliflower mosaic virus (CaMV), the template for transcription is a circular mini-chromosome. The genomic 35S RNA and the subgenomic 19S RNA use the same poly(A) addition site¹⁰. Regions equivalent to the retrovirus U3 and U5 are defined by the start site of (–) and (+) strand DNA synthesis during reverse transcription. In CaMV, 'U3' contains the 35S promoter from which the genomic RNA is transcribed and R contains all the elements necessary for 3'-end processing of the 35S and the 19S RNA.

was abolished on deletion of the consensus hexanucleotide AATAAA (ref. 9) in R (construct R-CAT Δ , Fig. 2b). A new band of 280 nucleotides was then obtained, the expected size for transcripts reading through R and being processed 20 nucleotides downstream of a cryptic AATAAA in the *nos* signal^{7,8}. This AATAAA is located 120 nucleotides upstream of the natural *nos* gene poly(A) addition site. Additional elements within the CaMV R are likely to be responsible for the recognition of this cryptic site in our experiments.

To measure the bypass of the CaMV polyadenylation site at the 5' end of the genomic RNA, we modified plasmid R-CAT by placing R immediately downstream of the 35S RNA promoter (the CaMV equivalent of U3, Fig. 1). On RNA mapping with

probe P1, we detected three bands of the expected size for transcripts processed at the CaMV poly(A) addition site and at the normal and the cryptic sites in the *nos* segment (plasmid R0, Fig. 2c, d). The proportion of transcripts processed at the CaMV versus the *nos* sites was used as a measure for the bypass of the first signal (Fig. 2e). About two-thirds of the transcripts were processed at the end of R and one-third at the two *nos* sites.

To determine whether the inefficient use of the CaMV poly(A) addition site in construct R0 was caused by its proximity to the RNA 5' end, a 40 nucleotide polylinker was placed at the beginning of R (construct R1). DNA fragments of increasing length, isolated from calf thymus DNA, were inserted into the polylinker creating constructs R2-R7 (Fig. 2c). Increasing the

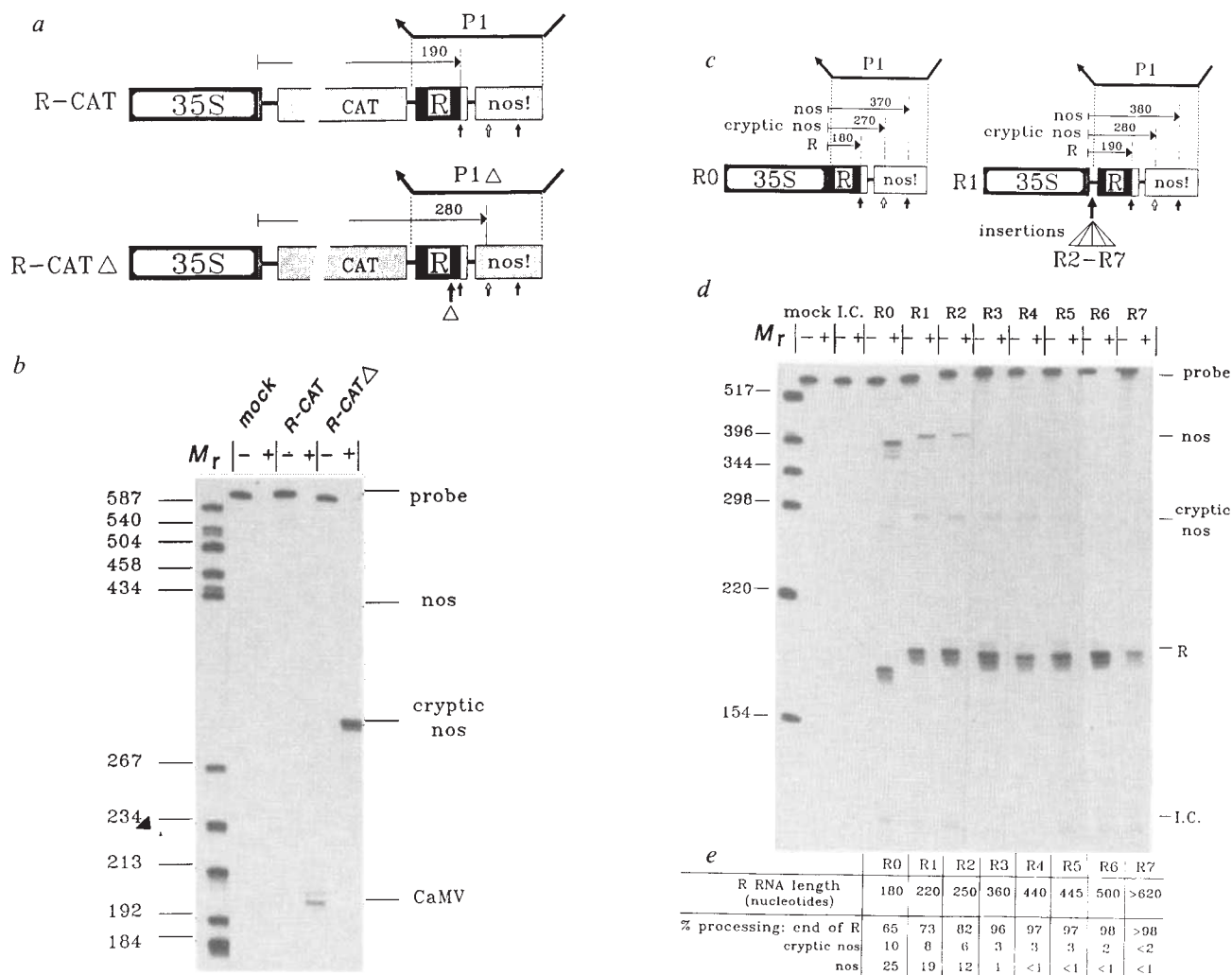


FIG. 2 Changes in recognition of the CaMV polyadenylation signal on increasing RNA length. **a**, c, RNA mapping strategy. The 35S promoter, CAT reporter gene, CaMV R plus 20 nucleotides of CaMV downstream sequences (empty box), and *nos* polyadenylation signal (nos!) are shown. All plasmids except R0 contain a 40-nucleotide polylinker (thick black line) between the third and fourth nucleotide of R, used for insertions. In construct R-CAT Δ , the AATAAA deletion is indicated (Δ). Normally used (small black arrows) and cryptic (small open arrow) 3'-end cleavage sites in R and *nos* fragments, transcripts (thin lines and arrows) and 300-nucleotide antisense probes P1 and P1 Δ containing part of the polylinker (upper thick line) are shown. Sizes of expected protected fragments are given. **b**, d, RNase protection assays. Lane M_r, relative molecular mass markers as ³²P-labelled single-stranded DNA fragments. Hybrids are shown before (–) and after (+) digestion. Lane 'mock', transfection in absence of input plasmids. **d**, A patchwork of several exposures of the same gel was assembled to compensate for variation in total RNA recovered. This is measured by comparing the intensity of the band (I.C.) corresponding to a cotransfected internal control plasmid containing a truncated R (without *nos* fragment), partly homologous to probe P1

(120 nucleotides). Lane I.C., transfection with internal control only. **e**, Quantification of transcripts produced. Percentage of processing: the ratio of the relevant band was determined by optical densitometry (the number of labelled nucleotides in each band was taken into account). Average numbers from this and three other independent experiments are given.

METHODS. DNA constructs: R-CAT, *EcoRI* (35S promoter and CAT gene) and *EcoRI*–*HindIII* (R) fragments of pDW2¹⁸ and *SacI*–*EcoRI* (nosPA) fragment of pBI 101 (ref. 19) into pC19R⁺ (ref. 20) polylinker; R-CAT Δ , deletion of AATAAA within R by oligonucleotide-directed mutagenesis; probes P1 and P1 Δ , *PstI*–*EcoRI* (R) and *EcoRI* (nosPA) fragments of R-CAT and R-CAT Δ into pGEM2 (Promega) polylinker; R0, *NcoI*–*SacI* fragment (6,909–7,632) of CaMV-S²¹ into *NcoI*–*KpnI* (blunted) fragment of R-CAT; R1, *EcoRI* (35S promoter and R) fragment of pDH51¹⁸ ligated to *EcoRI* fragment of R0, R2–R8, random calf thymus *Sau3A* DNA fragments into *BamHI* site of R1 polylinker. Protoplast transfection and RNA analysis: *N. plumbaginifolia* protoplasts were transfected and total RNA purified after 6 h and mapped by RNase protection as described^{22,23}. DNase treatment was included.

space between the transcription initiation and the CaMV poly(A) addition site resulted in an increased proportion of transcripts processed at the CaMV site (Fig. 2d and e). A pre-mRNA length of over 600 nucleotides resulted in <2% read-through of the CaMV signal. Sequencing of the inserts revealed no obvious homology to known consensus sequences beneficial for 3'-end processing. It is interesting that increasing the distance between the 35S promoter and the polyadenylation sites also resulted in progressively stronger recognition of the cryptic over the normal *nos* site (Fig. 2e). The data shown were obtained with *N. plumbaginifolia* (Solanaceae) protoplasts, which is not a host for CaMV infection. Similar results were obtained with *Orychophragmus violaceus* (Cruciferae) protoplasts, a CaMV host (data not shown).

If these results were to apply to natural CaMV infection of plants, then roughly two-thirds of the initiation events would be aborted by processing and polyadenylation at the 5' R element, leading to the production of a large amount of 'short stop' RNA. To test this, total RNA was purified from an infected turnip plant and mapped with a probe covering R and 3' and 5' flanking sequences (Fig. 3a). We expected protected fragments of 180 nucleotides for the short-stop transcript, 220 nucleotides for the 5' end of the 35S genomic RNA, and 260 nucleotides for the 3' ends of the 19S and 35S RNAs¹⁰ (Fig. 1). All three fragments were detected in both the total RNA and the poly(A)⁺ fraction. The 220-nucleotide fragment was, however, also detected in the poly(A)⁻ fraction, presumably as a result of degradation of the very long 35S RNA (8,200 nucleotides). As the short-stop RNA is present and polyadenylated in an infected plant, it will be interesting to investigate its possible role in CaMV infection.

To determine whether the spacing hypothesis for conditional recognition of a polyadenylation signal is relevant only for the CaMV system or reflects a more general feature of plant polyadenylation signals, the two signal-containing fragments were exchanged. The CaMV signal was then used to rescue the transcripts that bypassed the *nos* signal (Fig. 4a). Also, the efficiency of 3'-end processing at the *nos* site depended on its distance from the transcription start site (Fig. 4b). The three bands observed confirm the heterogeneity of the normal processing site described earlier⁷. Protected fragments corresponding to transcripts processed at the cryptic *nos* site were not detected in the lower part of the gel (data not shown).

Processing of the nascent RNA 3' end probably occurs before termination of transcription¹¹⁻¹⁴. On this basis, the progressive recognition of a polyadenylation signal with increasing pre-mRNA length could be explained by several mechanisms. One possibility is that the polyadenylation signal of a short transcript is poorly accessible to the processing/polyadenylation complex, perhaps as a result of steric hindrance caused by a capping enzyme bound to the 5' terminus of the nascent RNA¹⁵⁻¹⁶; or similarly, the processing/polyadenylation complex might require a long foothold upstream of the poly(A) addition site. Alternatively, one could imagine that the RNA polymerase II initiation complex is converted *en route* to an elongation complex by progressive gain or loss of factors, allowing or preventing recognition of the polyadenylation signal. Denome and Cole¹⁷ proposed that such a factor, either soluble or associated with the RNA polymerase, is involved in polyadenylation. Finally, a minimum mRNA length requirement could provide a simple explanation for the conditional recognition of some other retrovirus or retroelement polyadenylation signals. □

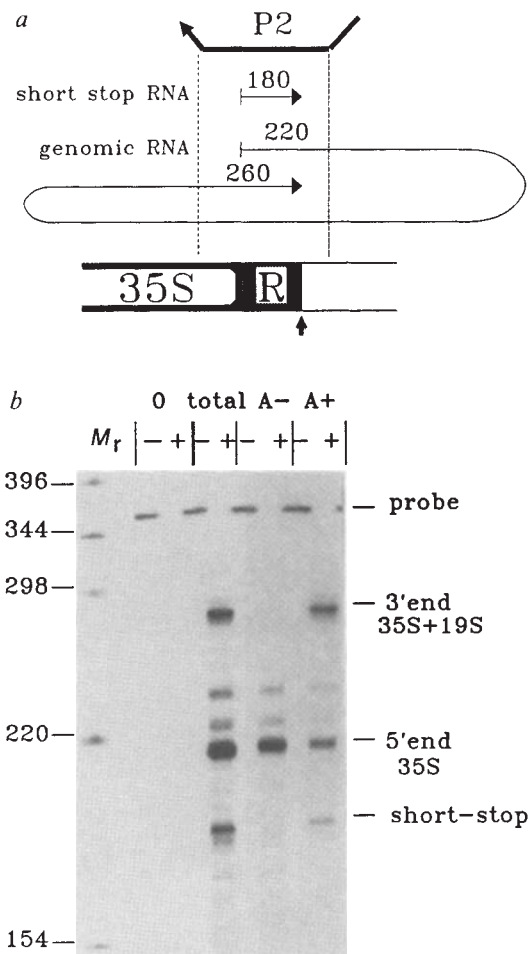


FIG. 3 Detection of the 'short-stop' transcript in an infected plant. *a*, RNA mapping strategy. Relevant CaMV regions (35S promoter and R), the antisense P2 probe and 35S genomic and short-stop RNAs with expected sizes of protected fragments are shown (as in Fig. 2). The 3' end of 19S RNA (not shown) would also give rise to a 260-nucleotide protected fragment. *b*, RNase protection assay as in Fig. 2. Control lane 0, 10 μ g transfer RNA was hybridized to probe P2; total, 1 μ g total RNA; A⁻, poly(A)⁻ fraction from 1 μ g total RNA; A⁺, poly(A)⁺ fraction from 1 μ g total RNA. METHODS. DNA constructs: Ca100, a CaMV hybrid strain containing the *Bst*EII-*Bam*HI small fragment from CM4184²⁴ and the small *Bam*HI and large *Bam*HI-*Bst*EII fragments from CaMV-S¹⁸ was used. Probe P2, *Eco*RV-*Bgl*II fragment of Ca100 (part of the 35S promoter, R and 40 nucleotides downstream) cloned into pGEM1 (Promega) polylinker. RNA analysis: total RNA was prepared 2 months after infection of the plant, essentially as described for protoplasts (Fig. 2). Poly(A)⁺ and poly(A)⁻ RNAs were fractionated by passage through an oligo(dT) cellulose column (Boehringer). RNA mapping was done as in Fig. 2.

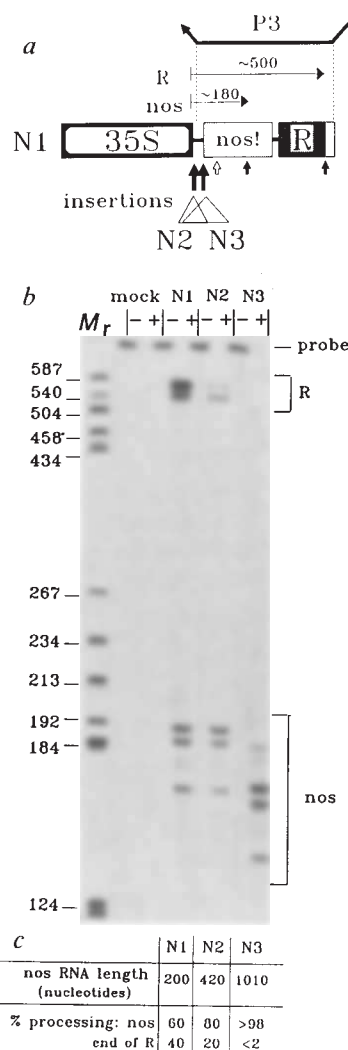


FIG. 4 Changes in recognition of the *nos* polyadenylation signal on increasing RNA length. **a**, RNA mapping strategy. The elements in each construct are represented as in Fig. 2. Two restriction sites in the polylinker (large black arrows) were used for insertion of sequences to produce N2 and N3. Because the antisense probe P3 (upper thick line) covers most of the polylinker, slightly smaller protected fragments are expected for N3. Sizes of expected protected fragments are indicated above each transcript. **b**, RNase protection assay, as for Fig. 2. **c**, Quantitation of transcripts produced. The *nos* RNA length, distance between transcription start site and normal *nos* processing site. Percentage of *nos* read-through (average of two experiments) was calculated by comparing R transcript intensity with *nos* transcript intensity and accounting for the number of labelled nucleotides in each band. **METHODS.** DNA constructs: N1, *EcoRI*-*PstI* fragment of pDH51¹⁸ (35S promoter), *PstI*-*HindIII* fragment of pNOSCAT²⁵ (*nos* polyadenylation signal) and *Bam*HI-*EcoRI* fragment of pDH51 (R) cloned into pC19R⁺ polylinker²⁰; N2, insertion of the calf thymus DNA used in R5 (Fig. 2) into *Bam*HI site of N1 polylinker; N3, exchange of the *EcoRV*-*PstI* fragment for that of pDW2¹⁸ (containing the CAT gene in *SalI* site of N1 polylinker); probe P3, *SmaI*-*EcoRI* fragment of N1 into pGEM2 polylinker (Promega). Protoplast transfection and RNA analysis as in Fig. 2.

- Proudfoot, N. J. & Brownlee, G. G. *Nature* **263**, 211-214 (1976).
- Guilley, H., Dudley, R. K., Jonard, G., Balazs, E. & Richards, K. E. *Cell* **30**, 763-773 (1982).
- Whitelaw, E. & Proudfoot, N. *EMBO J.* **5**, 2915-2922 (1986).
- Logan, J., Falck-Pedersen, E., Darnell, J. E. Jr & Shenk, T. *Proc. natn. Acad. Sci. U.S.A.* **84**, 8306-8310 (1987).
- Connelly, S. & Manley, J. L. *Genes Dev.* **2**, 440-452 (1988).
- Lanoix, J. & Acheson, N. H. *EMBO J.* **7**, 2515-2522 (1988).
- Bunick, D., Zandomeni, R., Ackerman, S. & Weinmann, R. *Cell* **29**, 877-886 (1982).
- Coppola, J. A., Field, A. S. & Luse, D. S. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1251-1255 (1983).
- Denome, R. M. & Cole, C. N. *Molec. Cell Biol.* **8**, 4829-4839 (1988).
- Pietrzak, M., Shillito, R. D., Hohn, T. & Potrykus, I. *Nucleic Acids Res.* **14**, 5857-5868 (1986).
- Jefferson, R. A., Kavanagh, T. A. & Bevan, M. W. *EMBO J.* **6**, 3901-3907 (1987).
- Marsh, J. L., Erfle, M. & Wykes, E. J. *Gene* **32**, 481-485 (1984).
- Frank, G., Guilley, M., Jonard, G., Richards, K. & Hirth, L. *Cell* **21**, 285-294 (1980).
- Vankan, P., Edoh, D. & Filipowicz, W. *Nucleic Acids Res.* **16**, 10425-10440 (1988).
- Goodall, G. J. & Filipowicz, W. *Cell* **58**, 473-483 (1989).
- Howarth, A. J., Gardner, J. C., Messing, J. & Shepherd, R. J. *Virology* **112**, 678-685 (1981).
- Fromm, M., Taylor, L. P. & Walbot, V. *Proc. natn. Acad. Sci. U.S.A.* **82**, 5824-5828 (1985).

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Identification of a 32K plasma membrane protein that binds to the myristylated amino-terminal sequence of p60^{v-src}

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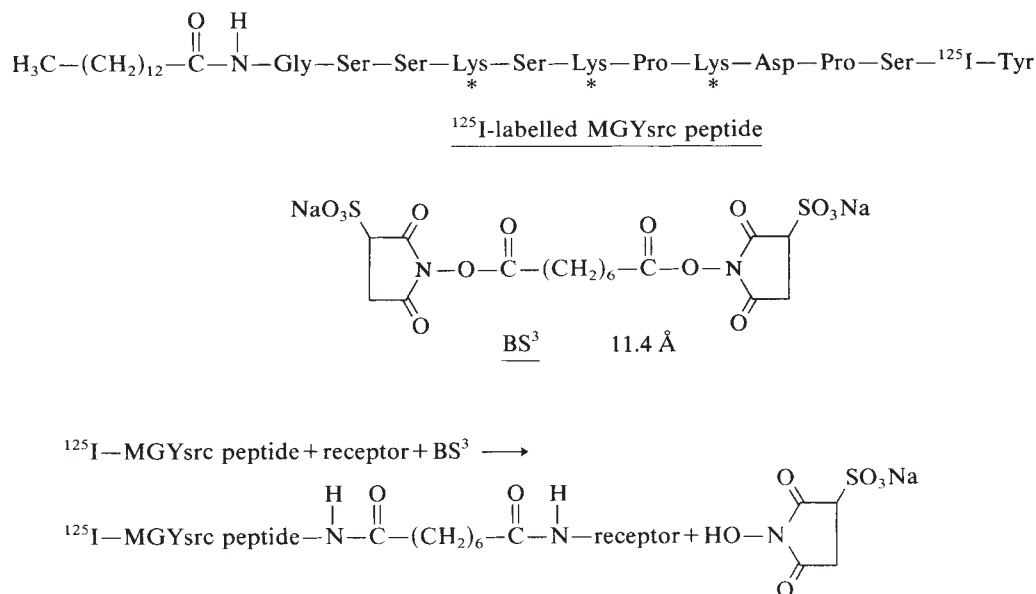
THE transforming protein of Rous sarcoma virus, p60^{v-src}, is a myristylated membrane-bound phosphoprotein¹⁻³. Interaction of p60^{v-src} with the plasma membrane is essential for transforming activity⁴⁻⁵, and is mediated by association with a membrane-bound Src receptor protein⁶. Evidence for the existence of an Src receptor is based on the ability of a myristylated peptide containing the N-terminal Src sequence to inhibit binding of p60^{v-src} to plasma membranes *in vitro*: binding of p60^{v-src} to a plasma membrane receptor is therefore mediated by N-terminal Src sequences⁶. Here we report that a myristyl-Src peptide, but not the corresponding non-myristylated peptide, can be specifically crosslinked to a plasma membrane protein of relative molecular mass 32,000 (*M_r* 32K). The 32K protein represents an Src-binding protein in the plasma membrane that is likely to be a component of the myristyl-Src receptor, and which could be involved in cellular transformation.

We have employed an N-terminal Src peptide to identify the Src receptor by chemical crosslinking according to the strategy outlined in Fig. 1. A myristylated Src peptide (MGYsrc) and the corresponding non-myristylated Src peptide (GYsrc) were synthesized⁷, consisting of amino acids 2-12 of the mature Src polypeptide sequence with a C-terminal tyrosine residue appended. Both peptides were iodinated on tyrosine with chloramine T to a specific activity of 5×10^5 c.p.m. μg^{-1} . The ¹²⁵I-labelled peptides were incubated with plasma membranes from uninfected vole fibroblasts, the bifunctional crosslinking reagent BS³ (Fig. 1) was added, and membranes were re-isolated by ultracentrifugation. When membrane-bound material was analysed by SDS-PAGE and autoradiography, a prominent iodinated band of *M_r* 33K was evident in membranes incubated with myristylated, but not with non-myristylated Src peptide (Fig. 2). Radio-label incorporation into the 33K band was inhibited by non-radioactive MGYsrc peptide, but not by GYsrc peptide (Fig. 2). In addition, myristyl-peptides containing the N-terminal sequences of other known myristylated proteins (cyclic AMP-dependent protein kinase⁸ and Gag polypeptide of murine leukaemia virus⁹) did not inhibit radiolabelling of the 33K band by iodinated MGYsrc (results not shown). The 33K band did

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- Temin, H. M. *Nature* **339**, 254-255 (1989).
- Stoltzfus, C. M. *Adv. Virus Res.* **35**, 1-38 (1988).
- Dougherty, J. P. & Temin, H. M. *Proc. natn. Acad. Sci. U.S.A.* **84**, 1197-1201 (1987).
- Benz, E. W. Jr, Wydro, R. M., Nadal-Ginard, B. & Dina, D. *Nature* **288**, 665-669 (1980).
- Seiki, M., Hattori, S., Hirayama, Y. & Yoshida, M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3618-3622 (1983).
- Bonneville, J. M., Hohn, T. & Pfeiffer, P. *RNA Genetics* Vol. 2 (eds Domingo, E., Holland, J. J. & Ahlquist, P.) 23-42 (CRC Press, Baton Rouge, 1988).
- Bevan, M., Barnes, W. & Chilton, M. D. *Nucleic Acids Res.* **11**, 369-385 (1982).
- Depicker, A., Stachel, S., Dhaese, P., Zambryski, P. & Goodman, H. M. *J. molec. appl. Genet.* **1**, 561-574 (1982).

FIG. 1 Crosslinking strategy. The homobifunctional N-hydroxysuccinimide ester, bis(sulphosuccinimidyl) suberate (BS³) was used to covalently crosslink iodinated myristyl-Src peptide (MGYsrc) to a membrane receptor by reaction with available amino groups. The MGYsrc peptide contains three potential sites for reactivity (epsilon amino groups of Lys, *). METHODS. Myristyl (MGYsrc) and non-myristyl (GYsrc) peptides, synthesized as described⁷ were iodinated on the C-terminal tyrosine residue with Na¹²⁵I and immobilized chloramine T (IODO-BEADS, Pierce), and were purified by chromatography through Sephadex G-10.



not represent an aggregate of crosslinked peptide alone, as no label was incorporated into a complex of this M_r in the absence of membranes (Fig. 2). Because the M_r of the MGYsrc peptide is ~1K, the M_r of the Src-binding protein detected in Fig. 2 is 32K.

Formation of a covalent complex between MGYsrc peptide and the 32K protein did not occur in the absence of a chemical crosslinker (Fig. 3a, lane 1), but was not limited to the use of BS³ as a crosslinking agent. A 33K crosslinked product was also produced when a disulphide-containing derivative of BS³, DTSSP, was employed (Fig. 3a, lane 5). As predicted, cleavage of the disulphide bond in DTSSP with free thiol resulted in dissociation of the complex between ¹²⁵I-labelled MGYsrc and the 32K protein (Fig. 3a, lane 4). Moreover, the heterobifunctional crosslinker MBS also resulted in attachment of ¹²⁵I-labelled MGYsrc to the 32K protein (Fig. 3a, lane 3). As MBS specifically crosslinks ligands containing primary amino groups to those containing sulphydryl groups, and the MGYsrc peptide contains no cysteine residues, the 32K membrane protein must contain a cysteine at or near the myristyl-peptide binding site. Finally, a similar 33K complex was formed between ¹²⁵I-labelled MGYsrc peptide and a 32K protein in membranes from vole fibroblasts transformed with Rous sarcoma virus (clone 1T) and from uninfected 3T3 fibroblasts, but not from membranes of *Escherichia coli* (Fig. 3b).

To better define the nature of the Src-binding protein, the 33K complex was analysed qualitatively and quantitatively. Formation of the 33K complex was sensitive to previous treatment of the membranes with trypsin (400 µg ml⁻¹ for 30 min at 37 °C; 60% reduction of binding) or heat denaturation (3 min at 90 °C; 95% reduction), confirming that the 32K binding component is a protein. In uninfected vole cells, the distribution of the 33K complex paralleled that of 5' nucleotidase, a plasma membrane marker enzyme¹⁰ (Fig. 4a). This agrees with previous results showing MGYsrc peptide inhibits binding of p60^{v-src} only in the plasma membrane fraction⁶. The amount of cross-linked MGYsrc peptide in the 33K complex was proportional to the amount of plasma membrane protein in the reaction (Fig. 4b). Moreover, addition of increasing amounts of ¹²⁵I-labelled MGYsrc to a constant amount of membrane protein demonstrated that formation of the 33K complex was saturable, with half-maximal binding occurring at ~6 µM peptide (Fig. 4c). These results indicate that binding occurs to a limited number of high-affinity sites, and is consistent with the interpretation that the 32K binding protein is a Src receptor. On the

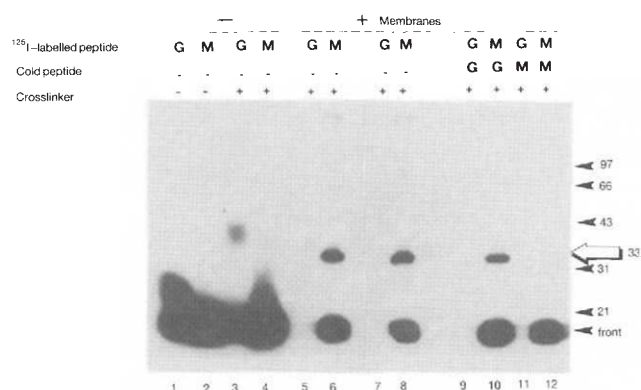
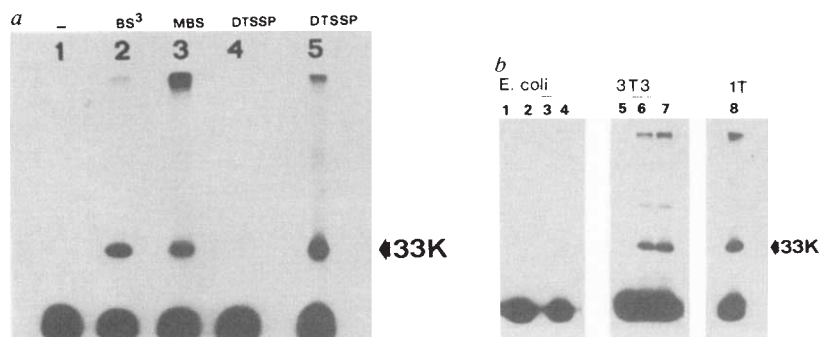


FIG. 2 Crosslinking of ¹²⁵I-labelled Src peptides to vole fibroblast plasma membranes. Odd-numbered lanes, ¹²⁵I-labelled GYsrc peptide. Even-numbered lanes, ¹²⁵I-labelled MGYsrc peptide. Lanes 1, 2, incubation in buffer; lanes 3, 4, incubation in buffer followed by 50 µg ml⁻¹ BS³ crosslinker. Reaction products from lanes 1–4 were run directly on the gel. Lanes 5, 6, incubation with membranes, followed by 50 µg ml⁻¹ BS³ crosslinker; lanes 7, 8, as lanes 5 and 6, respectively, except that the concentration of BS³ crosslinker was 100 µg ml⁻¹; lanes 9, 10, as lanes 7 and 8, except that 250 µg ml⁻¹ non-radioactive GYsrc peptide was added to the starting incubation mixture; lanes 11, 12, as lanes 7 and 8, except that 250 µg ml⁻¹ non-radioactive MGYsrc peptide was added to the starting incubation mixture. Only membrane-bound material is depicted in lanes 5–12; no 33K band was evident in the supernatant. The positions of the M_r markers (in K), as well as the dye front and the 33K complex are indicated in the right margin. Free peptide runs at the dye front. Little GYsrc peptide binds to the membranes, and consequently is not evident at the dye front in samples where membranes were re-isolated. A small amount of GYsrc became crosslinked in the absence of membranes (lane 3). This complex did not bind to membranes and migrated with a slower mobility than the 33K MGYsrc complex.

METHODS. Each reaction contained 100 µl HNE buffer (10 mM HEPES (pH 7.4), 100 mM NaCl, 1 mM EDTA) or uninfected vole (*Microtus agrestis*) P100 plasma membranes in HNE (1 mg ml⁻¹), isolated as described¹². Iodinated peptide (4×10^5 c.p.m. in 2 µl) was incubated with the membranes for 15 min at 20 °C, followed by 5 or 10 µl BS³ crosslinker (1 mg ml⁻¹ freshly prepared in dH₂O) for 15 min at 20 °C. Membranes were re-isolated by ultracentrifugation at 100,000 g for 15 min at 4 °C in a Beckman TL tabletop ultracentrifuge. The pellet was solubilized by sonication and boiling in SDS-PAGE sample buffer¹³, and released material analysed on a 10% acrylamide gel and autoradiography. Exposure was for 14 h at -80 °C with intensifying screen.

FIG. 3 The 33K complex can be formed with several different crosslinkers in plasma membranes from different species. *a*, Uninfected vole plasma membranes were incubated with ^{125}I -labelled MGYsrc, followed by incubation in the presence or absence of crosslinking reagent. Lane 1, no crosslinker; lane 2, $100\ \mu\text{g ml}^{-1}$ BS 3 ; lane 3, $100\ \mu\text{g ml}^{-1}$ *m*-maleimidobenzoyl-*N*-hydroxysulphosuccinimide ester (sulfo-MBS); lane 4, $100\ \mu\text{g ml}^{-1}$ 3,3'-dithiobis (sulpho-succinimidyl-propionate) (DTSSP); lane 5, as lane 4 except no reducing agent (β -mercaptoethanol) was added to the electrophoresis sample buffer. Higher M_r oligomers ($>300\text{K}$), which did not enter the separating gel, were sometimes apparent (lanes 3 and 5). Exposure was for 21 h at -80°C with intensifying screen. *b*, Lanes 1–4, *E. coli* membranes incubated with ^{125}I -labelled GYsrc (lanes 1 and 3) or ^{125}I -labelled MGYsrc (lanes 2 and 4) in the absence (lanes 1 and 2) or presence of $100\ \mu\text{g ml}^{-1}$ BS 3 . Lanes 5–7, 3T3 fibroblast plasma membranes incubated with ^{125}I -labelled MGYsrc in the absence (lane 5) or presence of $100\ \mu\text{g ml}^{-1}$ BS 3 (lanes 6 and 7). Lane 8, plasma membranes from Rous sarcoma virus-transformed vole fibroblasts (clone 1T) incubated with



^{125}I -labelled MGYsrc and $100\ \mu\text{g ml}^{-1}$ BS 3 .

METHODS. Each reaction contained $100\ \mu\text{l}$ membranes ($30\text{--}40\ \mu\text{g}$) prepared from mammalian cells as a P100 fraction¹² or from *E. coli* as a total membrane fraction by osmotic shock¹⁴. Reaction conditions were as described (Fig. 2).

basis of the data in Fig. 4c and the specific activity of the iodinated peptide, we calculate that there are $\sim 10 \times 10^6$ molecules of the 32K protein per cell, a number sufficient to accommodate the total number of p60^{v-src} molecules estimated to be localized to the plasma membrane ($1 \times 10^6\text{--}2 \times 10^6$ per cell^{11,12}).

One would predict that in cells transformed with Rous sarcoma virus, the number of available binding sites for ^{125}I -labelled

MGYsrc would be lower than in normal cells, as many of the potential binding sites would be occupied by endogenous p60^{v-src}. To test this hypothesis, a titration experiment identical to Fig. 4c was performed on plasma membrane fractions from transformed vole cells (clone 1T). Formation of the 33K complex was saturable, and half-maximal binding occurred at $6\ \mu\text{M}$ peptide. But the amount of 33K complex formed at saturation was 23% lower ($\pm 7\%$) than that detected in a parallel experiment with an identical concentration of nontransformed vole cell plasma membranes. Assuming the amount of plasma membrane protein is roughly the same in normal and transformed cells, and that the plasma membrane contains 10×10^6 molecules of the 32K protein, the presence of 2×10^6 molecules of p60^{v-src} would be expected to reduce MGYsrc peptide binding by 20%, a value that agrees well with the results presented above. As the N-terminal 12 amino acids of the cellular protein p60^{c-src} are identical to that of p60^{v-src}, the 32 K protein should also function as a receptor for p60^{c-src}. In uninfected cells, the calculated number of 32 K molecules is 100-fold greater than the number of p60^{c-src} molecules, implying that the 32 K protein may function to bind other proteins in addition to p60^{c-src}.

Taken together, these data indicate that the 33K band represents crosslinking of the myristylated Src peptide to a component of the plasma membrane Src receptor. Both fatty-acid and amino-acid sequence moieties of p60^{v-src} contribute to receptor recognition and binding. Receptor-mediated binding may therefore represent a general mechanism for localizing myristylated proteins to specific cellular compartments. We propose that the myristylated N-terminus of p60^{v-src} is recognized by and binds to the Src receptor, and thus becomes anchored to the plasma membrane. Proof that the protein identified in this study mediates receptor function awaits isolation, purification and reconstitution of the 32K protein. □

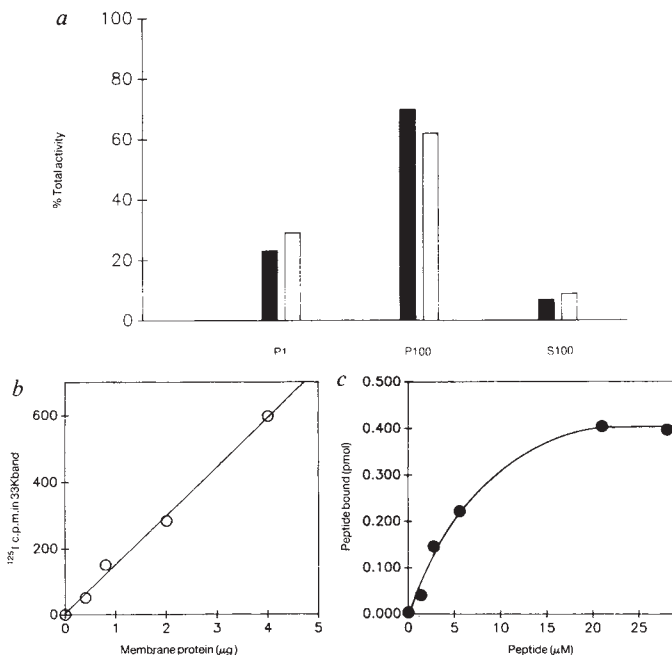


FIG. 4 Distribution and quantitation of the 33K complex. *a*, Crosslinking of ^{125}I -labelled MGYsrc to form the 33K complex (filled bars) and 5' nucleotidase activity (a marker for plasma membrane; open bars) were quantitated in nuclear (P1), plasma membrane-enriched (P100) and cytosolic (S100) fractions of uninfected vole fibroblasts. Each bar represents the average of duplicate experiments; standard deviations were $\pm 5\%$ or less. *b*, *c*, Formation of the 33K complex is dependent on the concentration of plasma membrane protein (*b*) and is saturable (*c*).

METHODS. *a*, Uninfected vole fibroblasts were fractionated in HEPES-containing buffers as described¹², and each fraction was resuspended in an equal volume of HNE. 5' nucleotidase activity was quantitated as described¹⁰. *b*, Each reaction contained the indicated amounts of uninfected vole fibroblast plasma membrane protein and 4×10^5 c.p.m. ^{125}I -labelled MGYsrc peptide. *c*, Each reaction contained $5\ \mu\text{g}$ uninfected vole fibroblast plasma membrane protein and the indicated concentration of ^{125}I -labelled MGYsrc peptide. Reactions were performed as described (Fig. 2). The 33K band was excised from the dried gel and counted in a gamma counter.

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- Brugge, J. S. & Erikson, R. L. *Nature* **269**, 346–348 (1977).
- Courtneidge, S. A., Levinson, A. D. & Bishop, J. M. *Proc. natn. Acad. Sci. U.S.A.* **77**, 3783–3787 (1980).
- Buss, J. E., Kamps, M. P. & Sefton, B. M. *Molec. cell. Biol.* **4**, 2697–2704 (1984).
- Cross, F. R., Garber, E. A., Pellman, D. & Hanafusa, H. *Molec. cell. Biol.* **4**, 1834–1842 (1984).
- Kamps, M. P., Buss, J. E. & Sefton, B. M. *Proc. natn. Acad. Sci. U.S.A.* **82**, 4625–4628 (1985).
- Resh, M. D. *Cell* **58**, 281–286 (1989).
- Deichait, I., Casson, L. P., Ling, H.-P. & Resh, M. D. *Molec. cell. Biol.* **8**, 4295–4301 (1988).
- Carr, S. A., Biemann, K., Shoji, S., Parmelee, D. C. & Titani, I. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6128–6131 (1982).
- Henderson, L. E., Krutzsch, H. C. & Oroszlan, S. *Proc. natn. Acad. Sci. U.S.A.* **80**, 339–343 (1983).
- Avruch, J. & Wallach, D. F. H. *Biochim. biophys. Acta* **233**, 334–347 (1971).
- Buss, J. E. & Sefton, B. M. *J. Virol.* **53**, 7–12 (1985).
- Resh, M. D. & Erikson, R. L. *J. Cell Biol.* **100**, 409–417 (1985).
- Laemmli, U. K. *Nature* **227**, 680–685 (1970).
- Osborn, M. J. & Munson, R. *Meth. Enzym.* **31**, 642–653 (1974).

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Weak-affinity chromatography

D. Zopf and S. Ohlson

Weak-affinity chromatography is a new method using readily reversible biospecific recognition as the basis for chromatographic separations.

A NOVEL approach to affinity chromatography has emerged in recent years, taking advantage of weak (readily reversible) biospecific recognition as the basis for chromatographic separations¹. Since its inception some 20 years ago, affinity chromatography has found application mainly as a small-scale, laboratory research operation². The standard scheme is basically an on/off procedure, using high-affinity (association constant, $K_{\text{ass}} > 10^5 \text{ M}^{-1}$) biomolecular recognition between the substance to be purified (the ligate) and the corresponding immobilized interactor (the ligand). The procedure has never gained wide acceptance either as a general analytical tool or in large-scale industrial operations, primarily because of high cost, low throughput, poor recovery of bioactivity, and lack of resolving power.

The stage has been set for a revolution in affinity chromatography by the simultaneous development of macroporous silica/polymer supports for HPLC³, highly efficient chemistries for coupling ligands, and ready access to multi-milligram amounts of proteins or peptide fragments produced by cellular or molecular cloning techniques. Instead of a process whereby substances are extracted from the mobile phase by virtue of high-affinity interactions with an immobilized ligand, the new approach uses weakly interactive systems where, typically, $K_{\text{ass}} = 10^2\text{--}10^4 \text{ M}^{-1}$. Porous matrices comprised of small particles ($D_p = 5\text{--}10 \mu\text{m}$) provide large available surface areas for ligand coupling, permitting ligand loads of, for example, proteins, up to 100 mg ml^{-1} column bed, while maintaining relatively short diffusion distances between active ligands. When purified and concentrated in an immobilized form, weak-affinity ligands can effect rapid and highly specific separation of ligates as retarded peaks under near physiological conditions, readily adaptable to quantitative measurement and experimental control.

Chromatographic perspectives

Weak-affinity chromatography based on biospecific recognition differs from other forms of interactive chromatography such as ion exchange and reversed phase only in the nature of the interaction between analyte and matrix, and is best understood in terms of basic chromatographic theory⁴. The capacity factor (k') that determines retention of a particular ligate is directly

related to the amount of active immobilized ligand (L) as well as to the affinity between the ligand and ligate, according to the following equation:

$$k' = C[L]K_{\text{ass}} \quad (1)$$

where C is a constant (with a value usually in the range 0.5–1.0) that reflects column features such as void fraction, porosity and density. Equation 1 is operative as long as the concentration of ligate at injection (c_0) is such that the product $K_{\text{ass}}c_0 \ll 1$, that is, the Langmuir adsorption isotherm for the system is within the linear range. It is worth noting that as affinity decreases, retention also decreases, but, at the same time, higher concentrations of injected ligate can be accommodated without loss of performance due to sample overload. Thus, in principle, weak-affinity chromatography favours rapid throughput and process scale-up. From equation 1, it is clear that significant retention ($k' = 1\text{--}10$) in a weak-affinity system depends upon a high concentration of active ligand coupled to the solid phase. For example, to achieve $k' = 1$ with an immobilized IgG monoclonal antibody ($M_r = 160 \text{ kD}$) at $K_{\text{ass}} = 10^3 \text{ M}^{-1}$ and $C = 1$, the required concentration of antibody on the column bed is approximately 75 mg ml^{-1} .

Weak-affinity separations

An example of chromatography on a 2-ml column loaded with 86 mg of monoclonal IgG coupled to tresyl-activated $10 \mu\text{m}$ porous silica particles is shown in Fig 1. Results from this and similar experiments confirm theoretical predictions that weak-affinity separations can have good chromatographic efficiency (narrow peaks and high plate numbers). Use of the weak-affinity approach overcomes the poor performance of traditional affinity chromatography, which is mainly due to slow desorption (desorption rate constant: $k_d \ll 1 \text{ s}^{-1}$). The chromatographic performance of weak-affinity chromatography actually improves as the strength of ligand/ligate interactions diminishes, in sharp contrast to traditional immunoassays and ligand-receptor binding assays, which are designed to perform with maximal efficiency for measurement of high-affinity ($K_{\text{ass}} > 10^6 \text{ M}^{-1}$) recognition events.

A striking prediction from theory is that weak-affinity chromatography has the potential to resolve completely several similar molecular species present in a

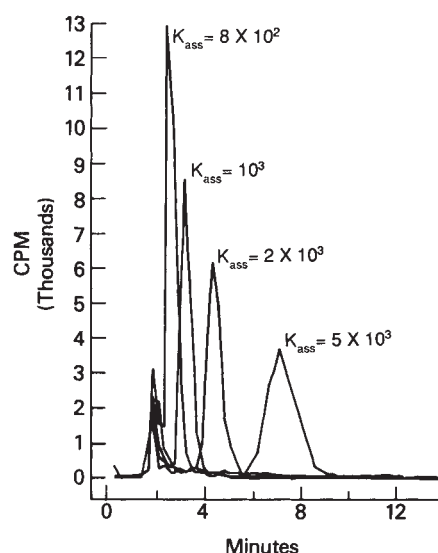


FIG. 1 Weak-affinity chromatography of the tritiated aldol of a glucose-containing tetrasaccharide, $(\text{Glc})_4$, on a 2-ml tresyl-activated SelectiSpher 10 macroporous silica HPLC column coated with IgG¹.

crude extract. Figure 2 shows computer-generated elution profiles for small molecular weight ligates with differing weak affinities ($K_{\text{ass}} = 10^2\text{--}5 \times 10^3 \text{ M}^{-1}$) for monoclonal IgG immobilized on an HPLC column. For this simulation, a suitable separation 'window' is defined over a range of capacity factors $k' = 1\text{--}12$. The

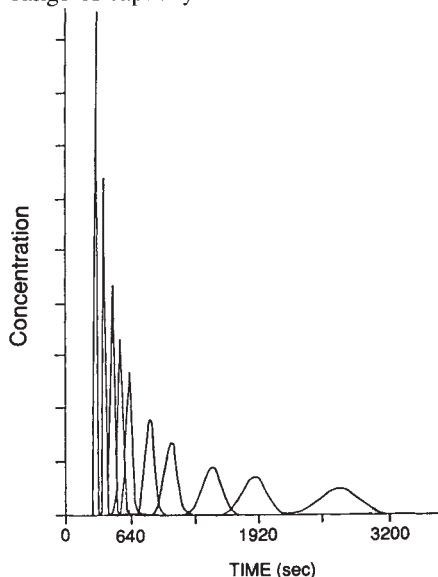


FIG. 2 Computer-generated chromatogram of weak-affinity chromatography of small ligates ($M_r = 600 \text{ D}$) on an HPLC column coated with immobilized monoclonal IgG antibody. K_{ass} varied between 10^2 and $5 \times 10^3 \text{ M}^{-1}$.

first and second peaks represent non-retarded and very weakly interacting species ($K_{\text{ass}} < 5 \times 10^3 \text{ M}^{-1}$) which, in practice, elute as a single 'void-volume' peak. The subsequent eight peaks illustrate the theoretical potential for weakly bound, 'cross-reacting' ligates to be separated isocratically by more than 90 per cent in less than one hour by HPLC. Figure 3 shows isocratic separation of the α - and β -*p*-nitrophenyl glycosides of maltose dissolved in a crude extract of fetal bovine serum using an HPLC column loaded with a monoclonal IgG antibody that binds the ligates with $K_{\text{ass}} = 10^3$ – 10^4 M^{-1} .

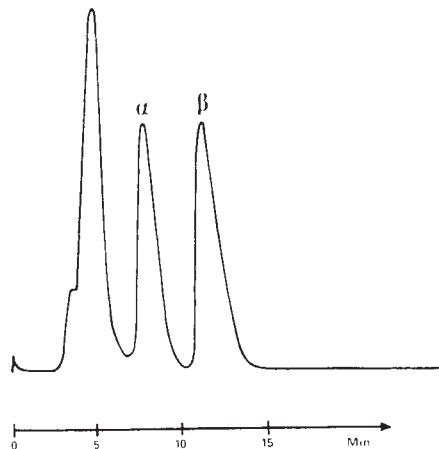


FIG. 3 Weak-affinity chromatography of the α - and β -*p*-nitrophenyl glycosides of maltose. 20 μl of sample containing 0.1 μg of each glycoside dissolved in a crude extract of 4 % fetal bovine serum was injected onto a 10 cm $\times$ 5 mm SelectiSpher 10 HPLC column coated with immobilized IgG monoclonal antibody. The column was eluted at 1 ml min^{-1} with 0.02 M sodium phosphate, 0.2 M sodium chloride, pH 7.5 at 45 $^{\circ}\text{C}$ and the eluate monitored for absorption of light at 300 nm.

Is weak binding specific?

The general observation that non-specific adsorption of solutes to chromatography support media is often of weak affinity does not support the converse statement that weak-affinity interactions are, by nature, non-specific. The specificity experienced in weak-affinity chromatography is the cumulative result of many serial weak-binding events whose kinetics, although readily reversible, depend upon binding energies between ligand and ligate that are significantly greater than the random weak interactions between biomolecules. The practical consequences of this specificity are evident in analyses for a specific oligosaccharide performed on crude samples such as human urine or serum⁷, where the analyte in such analyses appears as a single retarded peak, well-separated from hundreds of other compounds present at similar or much higher concentrations.

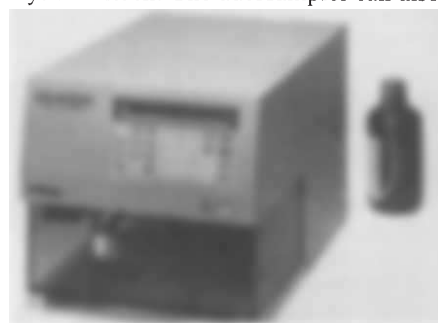
Future trends

As a research tool, weak-affinity chromatography is ideally suited to identification,

What's new in chromatography

A column designed to eliminate the problem of charge interaction and a preparative/analytical high-performance centrifugal partition chromatography system — highlights of this week's chromatography/HPLC feature.

For sample injection and complex liquid handling tasks, E. Merck recommends its LiChroGraph (AS-4000) **autosampler for HPLC** (Reader Service No. 101). Offering a 150 standard-vial capacity or 200 micro vials, the LiChroGraph provides three types of sample application: complete or partial filling of the loop, as well as micro-application for virtually zero sample loss, says E. Merck. The autosampler can also



be used to perform sample dilution steps before analysis, pipetting and mixing during pre-column derivatization. The addition of internal standards and the take-up and injection of a second phase in two-phase systems are additional features. All instrument functions, from simple needle movements to complex valve switching, are fully programmable. Run programs can be stored in the autosampler's memory. Full PC control of the instrument via a RS232C interface allows the storage of large numbers of application-orientated programs in the PC's memory.

isolation and systematic investigation of the components of weak bioaffinity reactions. For example, it may offer a means to define the targets recognized by 'lectin-like domains' of cell-surface glycoproteins isolated from membranes of interacting cells.

As a diagnostic tool, weak-affinity chromatography can measure multiple related, or (by combining appropriate ligands) multiple unrelated molecules in complex mixtures within a few minutes. In this respect, it promises to extend the application of immunoassays to families of highly similar molecules that are difficult to discriminate by traditional methods.

As a preparative tool, weak-affinity chromatography enables purification under mild buffer conditions, preserving the bioactivity of molecules. The potential of the weak-affinity method will be more fully realized when macroporous supports

Severn Analytical's SA 6160 **programmable autosampler** for HPLC has a 160-sample capacity and provides round-the-clock unattended analyses (Reader Service No. 102). The autosampler's injection mechanism is designed in such a way as to make the injection needle an integral part of the sample loop; this set-up eliminates carryover and ensures that the sample needle is continually flushed out with mobile phase. Injection volumes from 0.1 to 250 μl can be selected individually for each vial, with a reproducibility of better than 0.3 per cent standard deviation, says Severn. Using a hand-held control panel, users can program the SA 6160 with run parameters for run time, injection volume, number of injections and timed events. Parameters can also be changed during the run. Practical options include a thermostatted column compartment, column switching valves and a pre-column derivatization facility for amino acid analysis.

Accessories or necessities?

Plasti-Flex **chromatography columns** from Kontes with polycarbonate plastic barrels are designed to eliminate the problem of charge interaction between glass and some biomolecules (Reader Service No. 103). Stated applications for the columns include gel filtration, ion exchange, hydrophobic interaction and affinity chromatography. The columns are available in lengths ranging from 5 to 120 cm. Flow adapters can be supplied for columns

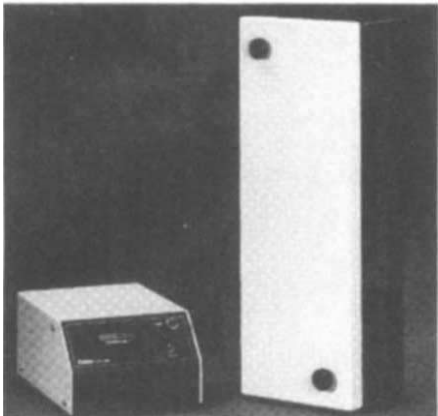
are coated with active fragments of macromolecules, such as variable-region, heavy-chain fragments representing weak-affinity monoclonal antibodies. This approach will permit significantly higher active ligand loads, improve preparative capacity and extend the usable affinity range. □

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1. Ohlson, S., Lundblad, A. & Zopf, D. *Analyt. Biochem.* **169**, 204–208 (1988).
2. *Affinity Chromatography — A Practical Approach* (eds Dean, P.D.G., Johnson, W.S. & Middle, F.A.) (IRL Press, Washington, D.C., 1985).
3. Ohlson, S., Hansson, L., Glad, M., Mosbach, K. & Larsson, P.O. *Trends Biotechnol.* **7**, 179–186 (1989).
4. Horvath, C. & Lin, H.J. *J. Chromatog.* **149**, 43–70 (1978).
5. Wang, W.T., Kumlien, J., Ohlson, S., Lundblad, A. & Zopf, D. *Analyt. Biochem.* **182**, 48–53 (1989).

of 1.0-, 1.5- and 2.5-cm diameter. Plasti-Flex columns are, says Kontes, typically used in aqueous mobile phases at pressures of one bar or less. For easy and secure connection to reservoirs and tubing, the column's polyethylene reservoir cap and bed-support cap have integral polypropylene luer-lock fittings.

For temperature control from 5 °C above ambient to 150 °C, Eldex Laboratories offers a new **chromatography column heater** (Reader Service No. 104). The heater can hold up to four 30-cm length columns plus guard columns. To



Eldex column heater — heats to 150 °C.

accommodate columns of all sizes, Eldex provides an assortment of interchangeable column inserts. The heater unit is insulated to protect the heater's outer casing and to maximize temperature stability, says Eldex. An injector valve can be installed for the preheating of samples.

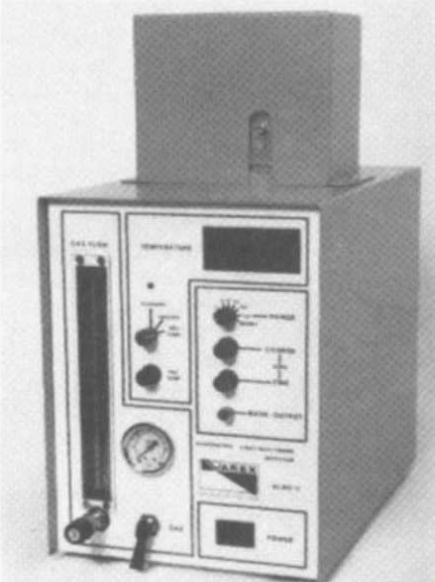
Solvent degassing, an important step for achieving clear chromatographic separations, need not be a problem with Knauer's **on-line degasser for HPLC** (Reader Service No. 105). Degassing is particularly important when using RI detectors, which are highly sensitive to undefined gas contents of solvents, says Knauer. Dissolved gases can cause micro bubbles in the pump and detector, which can result in baseline drift. Using Knauer's method, which the company claims is less expensive than helium degassing and more efficient than ultrasonic methods, the solvent to be degassed flows through a gas-permeable Teflon membrane system contained within a vacuum chamber. The pressure differential causes the gas molecules to escape through the Teflon membrane. According to Knauer, the slower the solvent passes through the membrane system, the more efficient the degassing process. Up to three channels with a flow rate of 5 ml min⁻¹ each can be used for either one gradient system or three isocratic systems.

Detection systems

Wyatt Technology has launched the new Wyatt/Optilab 903 **interferometric detec-**

tor with nanogram sensitivity in most HPLC applications (Reader Service No. 106). The 903 can be used for on-line operation by coupling it to any HPLC or GPC/SEC line, or any other light-scattering detector, to create a powerful macromolecular characterization system. Conventional RIs require recalibration after a solvent change is made because of temperature instabilities, but the 903 eliminates the problem by using a circulating water bath as a heat exchanger. Wyatt's 903 detector can also be used for off-line operation as a differential refractometer to determine the specific refractive index increment, dn/dc . In this case, it is used in conjunction with a light-scattering detector for the determination of absolute molecular weights of dilute polymer solutions.

Universal mass detection of surfactants, lipids, carbohydrates, polymers and petroleum additives can be achieved with the Vorex **laser light-scattering detector** for liquid chromatography — the LLSD MkII — from P.S. Instruments (Reader Service No. 107). Designed to link up to an LC separation system, the mass detector has stated applications in HPLC, gel permeation, size exclusion or supercritical fluid chromatography. The detector has, says PSI, particularly high sensitivity to



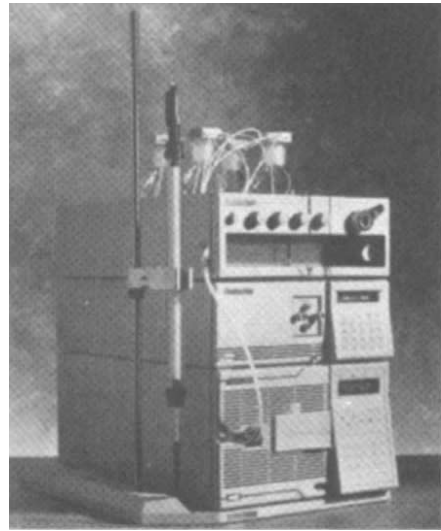
P.S. Instruments' general-purpose laser light-scattering detector.

compounds with specific functional groupings or chromophores. High stability and a 'noise-free' baseline performance in gradient elution analyses makes the LLSD MkII detector a useful general-purpose detector when compared with RI detectors.

Instrumental ideas

Hewlett-Packard, in extending its modular HPLC instrument range, has introduced **titanium HPLC systems** — HP 1050 Ti Series — designed for chemical

separations such as protein purification and ion chromatography that require the use of 'aggressive' mobile phases (Reader Service No. 108). The set-up includes a \$6,850 (US) isocratic pump, \$14,500 (US) gradient pump, \$1,300–1,710 (US) manual injection valve option, \$8,800 (US) auto-sampler and \$6,250 (US) variable-wavelength detector. Titanium alloy is used to



Titanium HPLC modules from HP are built to resist corrosive solvents.

replace all conventional stainless steel parts in the instrument. The machine is particularly useful where formic acid in the mobile phase is required for the purification of hydrophobic proteins. Other uses include the purification of biologically-active materials when exposure to iron ions could inactivate enzymes, and where high salt concentrations are necessary to achieve sufficient column selectivity. To prevent 'cracking' associated with stress corrosion of pure titanium, the HP 1050 series contains 0.2 per cent palladium. Although the use of titanium increases prices, this, says Hewlett-Packard, can be offset against the machine's high resistance to corrosion.

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Affinity Chromatography, located in the Isle of Man, has introduced an integrated PIKSI module (Protein Identification Kit by Sorbent Isolation) for purifying proteins by **affinity chromatography** (Reader Service No. 109). The module



ACL's PIKSI affinity chromatography module.

contains ten mimetic affinity ligands bonded to six per cent cross-linked agarose. This arrangement is designed to maximize the purification of most proteins in just a few minutes. No knowledge of the protein's molecular weight or isoelectric point is necessary. Mimetic ligand adsorbents are available in laboratory packs or in bulk quantities.

Sanki Laboratories is supplying a new modular system — the LLN-9 — for preparative/analytical **high-performance centrifugal partition chromatography** (HPCPC) (Reader Service No. 110). HPCPC uses liquid-liquid partition, countercurrent distribution to fractionate complex mixtures of chemical substances. Stated applications include the separation and purification of a broad range of synthetically and naturally-occurring chemical species, with particular use in the isolation of polar substances and biological materials — natural products, pharmaceuticals, biopolymers, antibodies and genetically-engineered molecules. As HPCPC does not use a solid stationary-phase support, irreversible retention of highly-retentive sample components is eliminated. Stationary-phase solvents are retained in



HPCPC: LC without a solid support.

the column by centrifugal force; this allows the use of high mobile-phase flow rates, without an appreciable loss of resolution, says Sanki.

The DX-100 is an integrated, single-channel **ion chromatograph** from Waters designed to perform all types of isocratic IC separations using conductivity detection (Reader Service No. 111). Waters says that for analysts considering purchasing an add-on conductivity detector to an HPLC system, the dedicated DX-100 complete IC system, with no extra pumps or valves needed, is a useful alternative. The DX-100 uses advanced conductivity detection and offers 11 operating ranges from 0.01 to 1,000 µs full scale. The 4,000 psi-rated solvent-compatible pump has a 0.5–4.0 ml min⁻¹ flow range with user-selectable high- and low-pressure alarms. All high-pressure flow paths are constructed of PEEK (polyetheretherketone) components for compatibility with the acids and bases used in IC. The £9,550 (UK) DX-100 IC system can be equipped with OmniPac and IonPac columns.

The PU4410 is the latest all-purpose **gas chromatograph** from Philips that is suitable for both capillary analyses and conventional packed-column chromatography (Reader Service No. 112). As the PU4410 is configured for increased throughput and ease of use, parameters are entered by a membrane keyboard, which is designed to guide the user through the process.



PU4410 GC system suits both capillary and packed-column analyses.

Operation is simplified still further with the stopwatch, flow calculator, auto-start and parameter-locking facility. Included in the package are two capillary injectors — one split-splitless, the other a programmable thermal vapouriser injector. Other injectors are available for all types of capillary, 530 µ and packed-column studies. Five types of detector are offered, optimized, says Philips, for capillary chromatography and offering fast response times and small dead volumes. In need of help, well Philips offers a chromatography customization service to cater for all user needs. □

These notes are compiled by Diane Gershon from information provided by the manufacturers. To obtain further details, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.